1882 Effects of Container Material and Soil Volume on *Rotylenchulus reniformis* and *Meloidogyne incognita* Population Development

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Abstract: The impact of pot material and soil volume on *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 population densities was evaluated in greenhouse tests. In all experiments, the treatments were arranged as a factorial, where (i) pot materials were clay, polypropylene, or polystyrene and (ii) soil volumes per pot were 90, 150, 250, 500, 750, or 1,000 cm^3. Based on the reproductive factor (RfF) values, polystyrene pots resulted in produced higher populations of *R. reniformis* than either the clay or polypropylene. Populations of *R. reniformis* in clay pots were 41% lower than those in polystyrene but 25% higher than those produced in polypropylene pots. The mean Rf values ranged from 65, 39, toand 29 in the polystyrene, clay and polypropylene pots, respectively, over all soil volumes.

The Rf values for *M. incognita* consistently increased with increasing soil volumes of 250 cm^3 and higher regardless of the pot material. The mean Rf values for clay, polypropylene, and polystyrene pots were 57, 44, and 43, respectively. The 150 cm^3 ConetainerCone-tainer™s produced greater numbers ($P \leq 0.05$) of *R. reniformis* vermiform life stages and eggs per gm of root than all 15 of the pot material and soil volume combinations. Opposite reactions were observed between *M. incognita* J2 and eggs. J2's declined while eggs per gm of root increased with increasing soil volumes. For each species we combined data across runs (20 replicates total) and resampled them 1000 times simulating experiments with 2 to 13 replicates to arrive at a 90th percentile upper limit for the coefficient of variation (CV). Six replications replicates resulted in a CV < 15% and <10% for *R. reniformis* and *M. incognita* respectively, thus this should be adequate to evaluate population increases in the greenhouse.

Keywords: *Confidence intervals, pot materials, greenhouse screening, Meloidogyne incognita, SAS® PROC GLIMMIX, reniform nematode, root-knot nematode, Rotylenchulus reniformis, soil volumes, standard errors, studentized residuals.*

The major plant-parasitic nematode pests of cotton (*Gossypium hirsutum* L.) are *Rotylenchulus reniformis* (Linford and Oliveira) and *Meloidogyne incognita* (Kofoid and White) Chitwood. *Rotylenchulus reniformis* has been found in every cotton-producing state east of New Mexico, and *Meloidogyne incognita* is found ininfests all cotton-producing states across the cotton belt in the US. (Lawrence and McLean, 2001).

Greenhouse screening procedures involving these nematodes are an important component in nematology research. The need for a standardized greenhouse nematode screening method has become apparent as greater emphasis has been placed on breeding cotton lines for resistance, while procedures for greenhouse evaluations remain unstandardized. Differences in pot type and soil volume may influence the final results and conclusions of experiments. In the recent literature, wide ranges of pot types and soil volumes have been used with numerous crops in greenhouse assays. Bouton et al. (1989) used 70 cm^3 polystyrene trays to screen alfalfa cultivars for resistance to *M. incognita*. Davis et al. (1996) evaluated selected soybean germplasm for resistance to *Meloidogyne* species,

Heterodera glycines, and *R. reniformis* in 15-cm-diam. clay pots. Robinson and Percival (1997) used 500 cm^3 of soil in 15-cm-diam. plastic pots to evaluate resistance of wild cotton accessions to *M. incognita* and *R. reniformis*. Fernandez et al. (2001) selected plastic pots containing 700 cm^3 of soil to evaluate induced soil suppressiveness to *M. incognita.* Cervantes-Flores et al. (2002) assessed resistance screening efficiency of sweet potato cultivars to *Meloidogyne* spp. using 400 cm^3 square pots and 150 cm^3 plastic ConetainerCone-tainerTMs. Diez et al. (2003) used 11.5-cm-diam. clay pots filled with 500 cm^3 of soil to evaluate competition of *M. incognita* and *R. reniformis.* Thus, previous research has been conducted with a wide variety of pots and soil volumes. In order to compare and rely on pot-screening results, the effects of these different factors should be known.

This study focuses on improving greenhouse screening methods in which nematodes are involved. Three common pot materials, clay, polypropylene, and polystyrene, were selected for the experiments. Clay pots are often standard greenhouse growth pots but require washing and sterilization as far as pathogens are concerned. Both processes are time consuming and expensive. Polypropylene pots are common in the greenhouse nursery industry but tend to have a short shelf life, require washing, and can not be heat sterilized. Polystyrene pots are available through the food service industry, are inexpensive and designed for one-time use.

The specific objectives of this study were to: 1) evaluate pot types and soil volumes in the greenhouse to determine their effects and interactions on *R. reniformis* and *M. incognita* population development; 2) determine the optimum number of replicates for these nematodes in greenhouse studies; 3) determine if polypropylene ConetainersCone-tainers™ (Stuewe & Sons, Inc. Corvallis, OR) are a realistic alternative to the standard greenhouse pots; and 4) determine objective rules based on which "true" outliers may be detected.

MATERIALS AND METHODS

Pot material and soil volume: Greenhouse experiments were conducted to evaluate the effects of soil volume and pot type on *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 populations. Separate experiments were conducted for both *R. reniformis* and *M. incognita* and each of these experiments was repeated once. In all four experiments, the treatments were arranged in a factorial design where pot materials were (i) clay, polypropylene, or polystyrene, and (ii) soil volumes per pot were 90 cm^3, 150 cm^3,

250 cm^3, 500 cm^3, 750 cm^3, or 1,000 cm^3. Polypropylene Ray Leach Cone-tainers™ (Stuewe & Sons, Inc. Corvallis, OR) at the 150 cm^3 soil volume was the only "extra" treatment in this augmented factorial treatment design. Each test thus contained 160 experimental units (150 for the complete factorial plus 10 Cone-tainers™) and was repeated once for a total of 320 experimental units and data values for every parameter measured for each per nematode species. Polypropylene Conetainers (Stuewe & Sons, Inc. Corvallis, OR) at the 150 cm^3 soil volume was the only "extra" treatment in this augmented factorial treatment design. The experimental design for each run was a randomized complete block with 10 replicates. The experiments were conducted in a greenhouse between March and September, where ambient temperatures ranged from 25°C to 32°C. The soil used in these experiments was classified a loamy sand (72.5% sand, 25% silt, 2.5% clay; pH 6.4) obtained from the Field Crops Unit of the E. V. Smith Research and Extension Center, Shorter, AL. Soil was collected from the top 20 cm of the soil profile and sieved to remove large particles. Soil was sterilized by autoclaving at 121°C and 103 kPa for 2 hrs on two consecutive days. *Rotylenchulus reniformis* and *M. incognita* populations were maintained

on a susceptible cotton cultivar, Paymaster 1218 BG/RR. Nematode inoculum was extracted from the soil by combined gravity screening (specific gravity = 1.13) and sucrose centrifugal floatation (specific gravity = 1.13) and enumerated with a stereo microscope (Jenkins, 1964). *Rotylenchulus reniformis* and *M. incognita* eggs were extracted from the roots by shaking for 4 min in a 0.6% sodium hypochlorite (NaOCl) solution (Hussey and Boerma, 1981).

Each test was planted with the cotton cultivar Paymaster 1218 BG/RR. Three seeds were planted in each individual pot. A border of 500 cm^3 polystyrene pots containing a single cotton plant each was placed around the test to reduce the potential of environmental influence.

Seven days after planting, pots were thinned to 1 seedling/pot. After thinning, a depression (1-cm-diam. and 5-cm-deep) was made approximately 1 cm away from the cotton seedling. A suspension of 1,000 *R. reniformis* juvenile and vermiform adults or *M. incognita* J2 and eggs was pipeted into each depression, which was then filled with soil to prevent desiccation. Tests were initialed weekly and ran concurrently with supplemental lighting (1000-W mental halide lamps with a 15-h photoperiod) and temperature range from 21 to 32 C.

Plants were grown in the greenhouse for 60 d following inoculation. Pots were watered manually as needed and fertilized weekly using Peters 20-10-20 water-soluble fertilizer (BWI, Jackson, MS). At harvest, vermiform life stages of the nematodes and eggs were extracted from the soil and plant roots as previously described. *Rotylenchulus reniformis* and *M. incognita* eggs were stained to facilitate counting using 20 ml of a 5% red food coloring (# 40) solution and microwaved for approximately 2 min and 35 sec. Cotton plant height, shoot fresh and dry weights, and root fresh and dry weights were also recorded.

Analysis of the complete factorial: Generalized linear models (GLM) methodology with the lognormal distribution function was employed to analyze the data (SAS 9.1, Cary, NC). These models extend mixed models methodology to include distributions from the exponential family of distributions. The normal (Gaussian) distribution and the lognormal distribution are members of this family. GLM models consist of three parts: (1) the random component, which deals with the conditional distribution of the dependent variables, given a set of independent predictors; (2) the linear function of the independent variables (the classical model statement); and (3) an invertible link function $g(\mu_i) = \eta_i$, which transforms the expectation of the response to the linear predictor (McCullagh and Nelder, 1990). What makes the GLM approach different from the traditional transformation approach to non-normal data is that the link function and the conditional distribution of the dependent variable are separated. Response variables were vermiform/J2counts per 100 cm^3 soil volume, eggs per gram root fresh weight, and the reproductive factor (Rf= final population/initial population). All counts were increased by 0.5 to avoid discarding zero counts. The pot materials factors (clay, polypropylene, and polystyrene) and soil volume (90, 150, 250, 500, 750, and 1,000 cm^3) and their interaction were considered to be fixed effects, whereas run or repeat experiments and blocks were random effects. Distributional characteristics of the datasets were assessed using the studentized residual graphics panel and fit statistics in SAS PROC GLIMMIX. The lognormal distribution provided the best fit for the current dataset. Once the distributional characteristics were ascertained, the effect of soil volume was modeled directly in PROC GLIMMIX by treating it as a covariate in the analysis. A backwards selection procedure was adopted beginning with the most complex probable model, which included the main effect for pot material and linear and quadratic

effects for soil volume plus their interactions (Littell et al., 20060). Non-significant terms ($P > 0.15$) were then excluded and the new model fitted. This process was repeated until all terms were significant at $P > 0.10$.

Removing extreme observations: Because 10 replicates were available for each pot material × soil volume combination in each of the four runs of the experiment, the effect of dropping the replicate with the highest count, lowest count, or both from the statistical analysis was then investigated. The approach taken in this study was to predict Rf for pot material at a given level of the covariate, chosen to be a standard soil volume of 250 cm^3.

Outlier detection: Discarding extreme observation without regard to statistical necessity is a wasteful practice that should be avoided. Studentized residuals can be a decision making tool to manage the elimination of extreme observations. Studentized residuals are created by dividing each residual by the overall standard deviation among residuals:

$$residual_i = \frac{\hat{e}_i}{\sqrt{\hat{\sigma}^2_i (1 - h_{ii})}}, where$$

e_i is the i^{th} residual, σ^2 is the estimated variance among residuals, and h_{ii} is the i^{th} diagonal element (ranging between 0 and 1) of the leverage (or "hat") matrix **H**. It measures the influence of the i^{th} observation in the matrix and helps to identify influential observations (Littell et al., 2006). Studentized residuals will approximate a normal distribution with mean 0 and variance 1 when residual degrees of freedom for a model get large. An observation was classified as an outlier if its studentized residual exceeded the value ± 3.0.

Number of replicates: For the evaluation of the number of needed replicates, the 20 observations for a given nematode species × life stage x pot material x soil volume combination were treated as coming from a single experiment. The SAS procedure SURVEYSELECT (http://support.sas.com/onlinedoc/913/docMainpage.jsp; verified 30. April, 2007) was then employed to resample the dataset 1000 times using sample sizes from 2 to 13. We then calculated treatment (pot material × soil volume) least squares means and associated standard errors for each replicate of the sampled dataset. From this we calculated the coefficient of variation defined as

CV = 100 * Standard Error / Treatment Mean.

From the 1000 replicate samples, the 90^{th} percentile for the coefficient of variation for sample sizes n = 2 to 13 was calculated.

ConetainersCone-tainers™ were compared: Lastly, we tried to determine how reliable nematode counts obtained from 150 cm^3 polyethylene ConetainersCone-tainers™ were compared to the other pot materials. In order to answer this question, we analyzed the dataset as having 16 treatments (3 pot materials × 5 soil volumes plus Conetainer Cone-tainers™), ignoring the augmented factorial structure, and calculated differences between the ConetainerCone-tainer ™ control and the remaining 15 treatments using Dunnett's test.

RESULTS

Pot material and soil volume: The simplest relationship between the pot material and soil volume was observed for the *R. reniformis* vermiform life stages (Figure 1, Table 1). The differences in *R. reniformis* vermiform life stage numbers standardized to 100 cm^3 over all soil volumes consistently decreased linearly with increasing soil volume in all three pot types. The equivalent performance in *R. reniformis* vermiform life stage numbers is evidenced by the common slope of the regression lines for all three pot materials (Fig. 1). The counts of vermiform life stages of *R. reniformis* from polystyrene pots were significantly ($P \leq 0.012$) higher than counts for clay and polypropylene pots (Table 2). The difference between clay and polypropylene was significant at $P = 0.073$. *Rotylenchulus reniformis* total vermiform life stages numbers averaged over all soil volumes ranged from 2314 for polypropylene, 3242 for clay, and 5359 for polystyrene pots (Table 3).

Rotylenchulus reniformis egg numbers per gram of root for pot materials fit a quadratic model with a linear and quadratic interaction between pot materials and soil volume (Table1, Fig. 1). The polystyrene pots at soil volumes from 250 to 1000 cm^3 contained more eggs per gram of root than clay and polypropylene pots. Linear and quadratic regression coefficient estimates for this pot material differed significantly ($P \leq 0.043$) from polypropylene but not from clay (Table 2). *Rotylenchulus reniformis* egg number ranged from 1444, 712, and 573 eggs per gram of root in the polystyrene, clay and polypropylene pots, respectively.

The relationship between pot materials and soil volume for *R. reniformis* Rf values was described with the most complicated model involving all interaction terms (Fig.1, Table 1). Quadratic regressions best illustrated the relationship between soil volume and *R. reniformis* Rf values (Table 2). Irrespective of pot material, Rf-values increased until at least 500 cm^3. Clay pots differed significantly ($P \leq 0.093$) from polypropylene pots for linear and quadratic regression coefficients; none of the other contrasts were significant. The mean Rf values ranged from 65, 39, and 29 in the polystyrene, clay and polypropylene pots, respectively, over all soil volumes.

The relationship between pot materials and soil volume for the *M. incognita* second stage juveniles (J2) involved a negative linear interaction between pot materials and soil volume (Table 1). Populations of *M. incognita* J2 when standardized to 100 cm^3 over all soil volumes decreased as soil volume increased for all pot materials (Fig. 1). Greater numbers of *M. incognita* J2 were found at the lower soil volume of 90 cm^3 in the polystyrene pot material as compared to polypropylene and clay. The regression for clay pots did not differ significantly ($P \geq 0.337$) from polyethylene pots (Table 2). The intercept for polystyrene pots was greater ($P \leq 0.003$) and the slope significantly steeper ($P \leq 0.073$) than either of the other two pot materials. The mean J2 count for polystyrene, clay, and polypropylene pots was 2119, 1236, and 1166 J2s per 100-cm^3 soil volume, respectively, over all soil volumes (Table 3). The slopes for the *M. incognita* J2 numbers *versus* soil volume in all three pot materials were steeper than those for *R. reniformis* vermiform life stages (Fig. 1, Table 2).

The relationship between the numbers of eggs per gram of root produced by *M. incognita* and pot materials and soil volume was similar to that produced by *R. reniformis* (Table 1). There was significant ($P \leq 0.0001$) linear and quadratic interaction between the pot materials and soil volume; thus, the relationship between soil volume and egg populations was best modeled by a separate quadratic regression for each pot material (Table 2, Fig.1). While the overall regression was significant, contrast failed to establish significant

differences between pot materials. A look at the graph in Figure 1 shows why this is the case. The mean egg production for polystyrene, clay, and polypropylene pots was 2807, 2392, and 1903 eggs per gram of root, respectively, over all soil volumes (Table 3).

The relationship between the Rf values produced by *M. incognita* and pot materials and soil volumes was simple (Table 1). There were no significant interactions between the pot material and soil volume. The relationship between soil volume and Rf values was best modeled by a quadratic regression with a common linear and quadratic term for all pot materials; hence, the regression lines in Figure 1 are parallel. Similar to what was seen with the *R. reniformis* data, Rf values for *M. incognita* consistently increased with increasing soil volume up to a certain point regardless of the pot material. However, soil volumes greater than 500 cm^3 did not further increase Rf values. The mean Rf value for clay, polypropylene, and polystyrene pots was 57, 44, and 43, respectively (Table 3).

Removing extreme observations: As might be expected, removal of the replicate with the maximum observation for either vermiform (J2) or egg number decreased the average Rf, whereas removal of the lowest replicate increased it (Fig. 2). However, the change was small, ranging from 97 – 107% compared to using all replicates. Whereas the relationship between removal of observations and magnitude of the mean was predictable and consistent for both species and stages, standard errors decreased in 9/12 cases. If there was an increase it was 3% or less. The maximum decrease in the standard error (9%) for Rf was obtained when the observation with the maximum egg number for *M. incognita* was removed from the analysis. The effect of removal of extreme observations on the differences between treatment means was even less predictable, but the effects were much larger than for means with relative differences ranging from 82% when both maximum and minimum observations were removed for *M. incognita* eggs to 134% when the maximum egg count for *M. incognita* was removed (Fig. 2). The effect of removing extreme observations on standard error of the differences was much smaller, ranging from 96 – 115%. The lack of a clear pattern when extreme values were removed from the dataset reinforces our contention made in the introduction that discarding maximum and/or minimum observations without regard for statistical necessity is a wasteful practice, particularly since there seem to be no predictable benefits.

Outlier detection: The fit of the regression models as judged by Generalized Chi-Square / degree of freedom ratio was quite good (Table 1); a ratio of 1.0 is the target for optimal fit. Hence using studentized residuals in SAS PROC GLIMMIX for outlier detection (> 3 SD) resulted in far fewer observations being discarded from the analysis compared to simply eliminating maximum and/or minimum observations (Table 1). Most outliers were detected for *M. incognita* (3/300 J2; 1/300 eggs, and 1/300 Rf) than for *R. reniformis* (1/300 eggs and 1/300 Rf), and all but one were concerned with the minimum observation. This reflects far fewer 'discarded' observations than the maximum-minimum approach, where either 60 (max. and min. eliminated) or 30 (either max. or min. eliminated) would have been eliminated without regard to the magnitude of the residual. All observation removal had studentized residuals ≤ 3.25, thus the effect of their removal was very small. Again, removal of "extreme" observations using the maximum and/or minimum criterion as compared to the studentized residual criterion ≥ 3.0 is a wasteful practice.

Number of replicates: Treating the 10 replicates for each of the two runs for a given species as coming from a single experiment in the sampling study would tend to maximize the residual error. We thus adopted the 90^{th} percentile for CVs as our evaluation criterion. The effect of the number of replications replicates on *R. reniformis* vermiform life stages and *M incognita* J2's and Rf values for both species was consistent over all pot materials and pot

sizes (Fig. 3). Six replicates in a greenhouse test resulted in a coefficient of variation (CV) < 20 and 10% for *R. reniformis* Rf values and vermiform life stages (Fig. 3). This number should be adequate to evaluate *R. reniformis* population increases. The CV for *M. incognita* was always lower than that observed with *R. reniformis*. Eight replications replicates in a greenhouse test resulted in a coefficient of variation (CV) < 10% for *M. incognita* Rf values and J2 life stages (Fig. 3).

How do ConetainersCone-tainers™ compare? The 150-cm^3 polypropylene ConetainerCone-tainer ™ produced greater numbers ($P \leq 0.05$) of *R. reniformis* vermiform life stages per 100 cm^3 and eggs per gm of root as compared to the clay, polypropylene, and polystyrene pot materials (Table 3). Fourteen of the 15 pot materials by soil volume combinations produced lower ($P \leq 0.05$) numbers of vermiform life stages per 100 cm^3 as compared to the ConetainerCone-tainer.™. ConetainersCone-tainers™ produced greater ($P \leq 0.05$) numbers of eggs per gm of root that all 15 of the pot materials by soil volume combinations. Rf values were greater in the ConetainersCone-tainers™ ($P \leq 0.05$) as compared to 90 and 1000 cm^3 clay pots, 90, 250, 500, and 750 cm^3 polyethylene pots, and 90 cm^3 polystyrene pots. The 150-cm^3 ConetainerCone-tainer ™ produced higher ($P \leq 0.05$) or similar numbers of *M. incognita* J2 in 14 of the 15 pot material and soil volume comparisons (Table 3). Only the 90 cm^3 polystyrene pots produced greater ($P \leq 0.05$) numbers of *M. incognita* J2 as compared to the ConetainerCone-tainer.™. The numbers of *M. incognita* eggs per gram of root were lower ($P \leq 0.05$) in 7 of the than the 15 pot material soil volume comparisons, with none being significantly greater than the ConetainerCone-tainer.™. ConetainersCone-tainers™Pots produced lower Rf values for *M. incognita* as compared to the clay, polypropylene, and polystyrene pot material in 12 of 15 comparisons.

DISCUSSION

A general pattern emerges from this analysis. *Rotylenchulus reniformis* vermiform life stages, eggs per gram of root and Rf values are generally highest for polystyrene pots, followed by clay and polypropylene pots. ConetainersCone-tainers™ allowed for optimal reproduction of *R. reniformis* producing greater Rf values than the all pot materials at similar soil volumes. Thus, ConetainersCone-tainers™ are ideal for screening large numbers of plants optimizing space limitations. *Meloidogyne incognita* race 3 second stage juvenile numbers, eggs per gram of root and Rf values were not consistently favored by any pot material. Larger volumes of soil produced greater numbers of J2's and larger Rf values. Pots allowed for adequate reproduction of *M. incognita;* however, this nematode did not increase to the levels that were observed with *R. reniformis*. One can only speculate on the reasons for the observed patterns, but it may have to do with changes in soil temperature and moisture over the course of a day. The insulating property of the pot material increases in the order: polypropylene < clay < polystyrene. Thus, one would expect polystyrene to be more stable over the course of a day than the other materials. Maintaining a constant moisture level in each pot is important in nematode reproduction (Rich et al., 1978). In our observations, clay pots tended to dry out more rapidly than polystyrene pots. Polypropylene pots produced the lowest vermiform and egg population densities compared to clay and polystyrene. On the other hand, variability was less in the polypropylene pots due to lower numbers of vermiform life stages, eggs per gm of root, and Rf values for *R. reniformis.*

Population densities of *R. reniformis* vermiform life stages and *M. incognita* J2 per 100 cm^3 were observed to decrease as pot size or soil volume increased. The increased space within the root system could potentially explain the reduced population size. Higher population densities occurred in the smaller pots in which root densities were greater; thus, the

nematodes were proficient in parasitizing the roots. Consequently, low volume polystyrene and 150-cm^3 ConetainersCone-tainers™ serve as an efficient, consistent, space-saving potting medium for *R. reniformis* and *M. incognita* greenhouse screening evaluations. The increased population numbers for *R. reniformis* in ConetainersCone-tainers™ is of particular importance in resistance screenings, where low counts would indicate resistance or tolerance. Utilizing ConetainersCone-tainers™ tends to minimize greenhouse space, which is always at a premium.

In our study, numbers of *M. incognita* were similar in the 150-cm^3 ConetainerCone-tainer ™ and 250-cm^3 clay, polypropylene and polystyrene pots. However, the numbers of *R. reniformis* vermiform life stages and eggs were greater ($P \leq 0.001$) in the 150-cm^3 ConetainerCone-tainer.™. The root systems of sweet potatoes grown in ConetainersCone-tainers™ were thicker, longer, and less dense that those formed in 400-cm^3 square polypropylene pots (Flores et al., 2002). The cotton root elongates rapidly (Smith and Cothren, 1999), placing the apical meristem tissues at the base of the ConetainerCone-tainer,™, 22.9 cm from the point of inoculation within 3 to 5 days after planting. *Rotylenchulus reniformis* will enter the root and initiate syncytia formation along the entire root surface (Jones and Dropkin, 1975; Diez, et al., 2003), while *M. incognita* selects the apical meristem root tissues for giant cell induction (Dropkin and Nelson, 1960; Diez et al., 2003). These differences in colonization and feeding preferences could explain the differences in population increases between the two nematodes in the 150-cm^3 ConetainerCone-tainer.™. The root architecture was limited by the narrow cone shape of the 2.54 × 22.9-cm ConetainerCone-tainer,™, which leads to long thin roots with root tips at the base of the ConetainerCone-tainer.™. *Rotylenchulus reniformis* non-selectivity in root tissues colonization and the limited migration distance imposed by the area of the ConetainerCone-tainer ™ contributed to the greater Rf values and vermiform life stage population densities produced within the ConetainerCone-tainer ™ as compared to 8 and 13 of the 15 pot material × soil volume combinations, respectively. Thus, the selective root colonization of *M. incognita* and the greater migration distance to the apical meristem tissues imposed by the ConetainerCone-tainer ™ probably restricted population densities of *M. incognita.* The ConetainerCone-tainer ™ did not produced higher *M. incognita* Rf values as compared to the 15 pot material × soil volume combinations.

We have shown that a criterion based on studentized residuals is preferable to simply discarding maximum and/or minimum observations for a given treatment. Although the practice of discarding outlier observations is rarely acknowledged in the literature, and thus hard to document, it nevertheless occurs. The advantage of this approach is twofold: (1) a reduction in the number of 'wasted' observations and (2) limits that can be set by the researchers, e.g., 2 standard deviation (SD) *vs*. 3 SD or 4 SD *vs*. 3 SD, based on professional experience with the particular screening system (e.g., host species, nematode species). As an aside, no observation would have been discarded in our experiment had we set the limit at 3.25 rather than 3.0.

In agronomic research, the number of replicates needed is of utmost importance in greenhouse and field tests. In both types of experimentation, space is usually limited. Increased numbers of experimental replicates and larger pots are generally thought to result in lower coefficients of variation (CV) in the greenhouse; however, larger pots take up more space and result in fewer replicates or treatments. In our study, we found that the lower volume pots (< 500 cm^3) were preferred over the higher volumes (> 500 cm^3) for increased populations of *R. reniformis* but not *M. incognita* in the 60 day test duration. From a population dynamics viewpoint, the significance of the reduction in the CV can easily be appreciated, as these differences tend to increase with increased populations. Six

replications replicates in a greenhouse test resulted in a CV < 15% (Fig. 4C). This number should be adequate to evaluate *R. reniformis* population increases. The CV for *M. incognita* was always much lower than that of *R. reniformis*. Smaller CVs optimize the ability of a study to detect differences between treatments in experiments. Conversely, since smaller pots are feasible and six replicates are adequate, the researcher could add another factor or level to an experiment, thereby increasing the scope of inference.

Finally, as shown by Weaver et al. (2007) generalized linear models enable the researcher to choose a conditional distribution from the exponential family that is appropriate for the data at hand rather than making an *a priori* assumption of using the lognormal or log transformation when a negative binomial distribution would make more sense because the data were over-dispersed.

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Table 1. Significance of fixed effects using the analysis of covariance approach to estimate regression parameters with the SAS procedure GLIMMIX with a lognormal distribution function. For each response variable we first fitted a full interaction model up to a 2nd order polynomial. Non-significant terms (P > 0.10) were removed and the most parsimonious model fitted. In a final step, outliers with a studentized residual > 3.0 were removed from the dataset before refitting the final model.

	*R. reniformins*Reniform				*M. incognita*Rootknot		
Source of variation	Vermiform	Eggs	Rf		J2	Eggs	Rf
Pot Material (PM)	< 0.0001	< 0.0001	< 0.0001		< 0.0001	< 0.0001	< 0.0001
Soil volume linear (SVL)	0.0002						< 0.0001
PM x SVL		0.0007	< 0.0001		< 0.0001	< 0.0001	
Soil volume quadratic (SVQ)							< 0.0001
PM x SVQ		0.0123	< 0.0001		0.0032	< 0.0001	
Gener. Chi-Square / DF - all observations	1.75	1.44	0.94		0.61	0.83	0.65
Gener. Chi-Square / DF - w/o outliers	1.75(0)†	1.40(1)	0.89(1)		0.55(3)	0.83(1)	0.62(1)

† Number of outliers removed is given in parenthesis.

Table 2. Estimates of regression coefficients, associated standard errors, and contrasts among pot materials for the graphs in Figure 1.

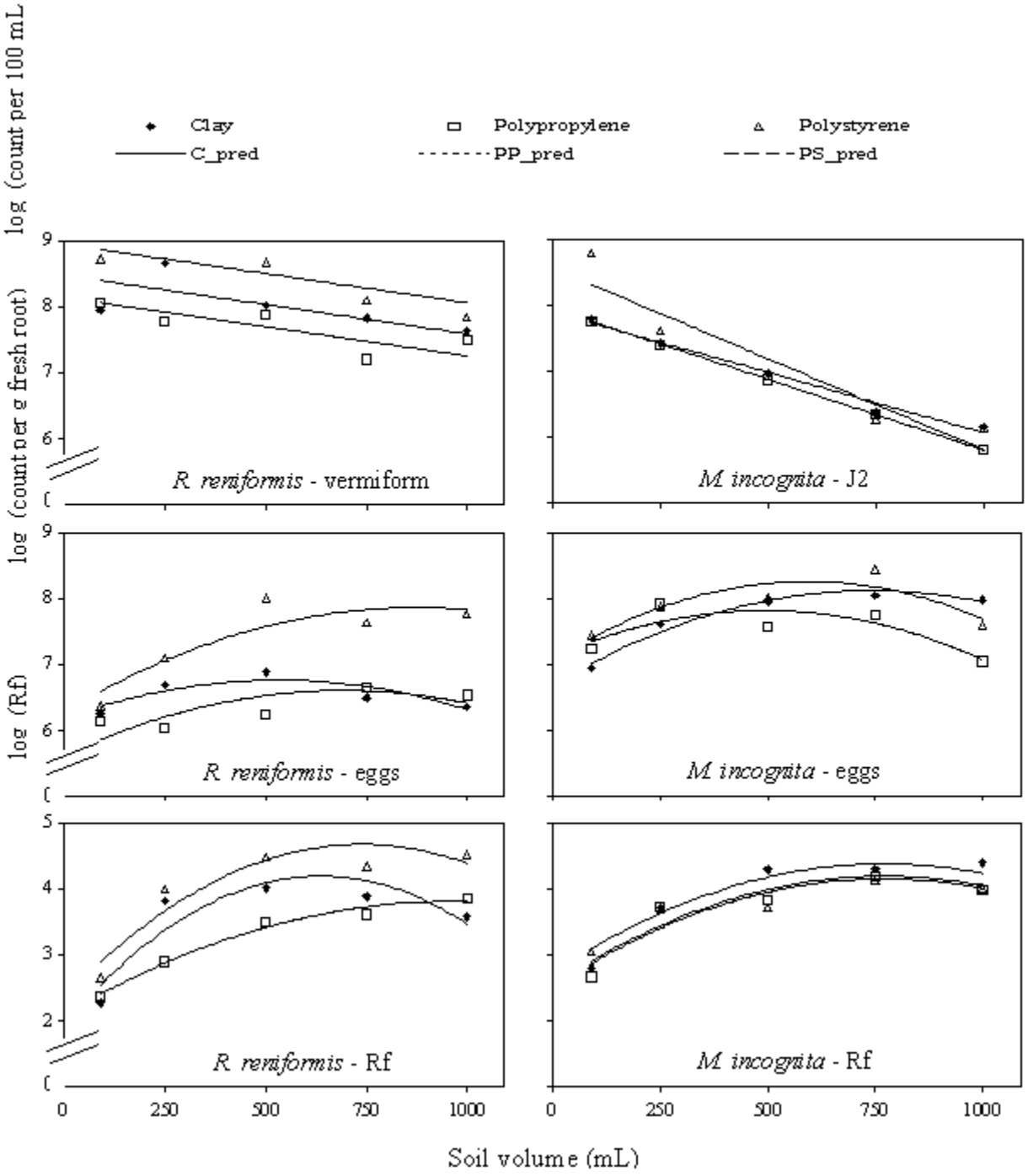
	Regression coefficient estimates				Contrast *P*-values		
Term	Clay (C)	Polypropylene (PP)	Polystyrene (PS)	SE	C vs. PP	C vs. PS	PP vs. PS
***R. reniformis* - vermiform**							
Intercept	8.48	8.14	8.95	0.180	0.073	0.012	<0.0001
Linear	-0.00089[†]			0.000232			
***R. reniformis* - Eggs**							
Intercept	6.14	5.79	5.96	0.346	0.454	0.703	0.710
Linear	0.002	0.001	0.006	0.002	0.424	0.155	0.028
Quadratic	-0.0000023	0.0000001	-0.0000038	0.000001	0.209	0.432	0.043
***R. reniformis* - Rf**							
Intercept	1.90	1.87	2.35	0.278	0.931	0.228	0.201
Linear	0.007	0.005	0.006	0.001	0.093	0.519	0.317
Quadratic	-0.000006	-0.000003	-0.000004	0.000001	0.038	0.315	0.280
***M. incognita* - J2**							
Intercept	7.8859	7.9423	8.5562	0.1741	0.784	0.002	0.003
Linear	-0.00182	-0.00214	-0.00275	0.000243	0.337	0.007	0.073
***M. incognita* - Eggs**							
Intercept	6.68	7.11	7.06	0.306	0.253	0.323	0.906
Linear	0.00389	0.00288	0.00405	0.00125	0.556	0.925	0.502
Quadratic	-0.000003	-0.000003	-0.000003	0.000001	0.610	0.751	0.252
***M. incognita* - Rf**							
Intercept	2.73	2.50	2.54	0.165	0.047	0.105	0.726
Linear	0.004298[†]			0.000624			
Quadratic	-0.000003[†]			0.000001			

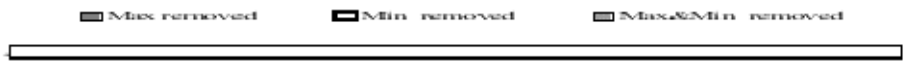
[†] A common regression line was fit for all pot materials.

Table 3. Comparing counts for ConetainersCone-tainers™ to other pot materials and soil volumes based on Dunnet's test. The number *for R. reniformis* vermiforms and *M. incognita* J2s are given in count per 100 mL soil. Egg counts are given as number of eggs per g of root fresh weight. The reproductive factor is unitless .

Pot	Soil	*R. reniformis*						*M. incognita*					
material	volume	Vermi.	Prob > t	Eggs	Prob > t	Rf	Prob > t	J2	Prob > t	Eggs	Prob > t	Rf	Prob > t
ConetainerCone-tainerTM	150	12817		12284		72		1859		3533		19	
Clay	90	2839	< 0.001	528	< 0.001	9	< 0.001	2400	0.326	1034	< 0.001	16	0.499
Clay	250	5751	0.045	807	< 0.001	45	0.122	1670	0.684	2008	0.053	40	0.006
Clay	500	3056	< 0.001	978	< 0.001	55	0.367	1056	0.030	2863	0.463	73	< 0.001
Clay	750	2505	< 0.001	667	< 0.001	48	0.180	584	< 0.001	3131	0.673	73	< 0.001
Clay	1000	2057	< 0.001	578	< 0.001	35	0.019	471	< 0.001	2926	0.511	81	< 0.001
Polyethylene	90	3290	< 0.001	464	< 0.001	10	< 0.001	2338	0.378	1384	0.001	14	0.218
Polyethylene	250	2482	< 0.001	421	< 0.001	18	< 0.001	1636	0.623	2730	0.369	41	0.005
Polyethylene	500	2625	< 0.001	517	< 0.001	32	0.008	957	0.011	1931	0.036	45	0.001
Polyethylene	750	1384	< 0.001	773	< 0.001	37	0.024	570	< 0.001	2319	0.143	65	< 0.001
Polyethylene	1000	1786	< 0.001	689	< 0.001	47	0.148	330	< 0.001	1149	< 0.001	53	< 0.001
Polystyrene	90	6195	0.068	590	< 0.001	14	< 0.001	6535	< 0.001	1702	0.015	21	0.784
Polystyrene	250	8865	0.355	1208	< 0.001	54	0.337	2031	0.733	2652	0.317	40	0.006
Polystyrene	500	5911	0.053	2985	< 0.001	88	0.491	1030	0.024	3005	0.572	40	0.005
Polystyrene	750	3287	< 0.001	2090	< 0.001	77	0.824	530	< 0.001	4662	0.334	61	< 0.001
Polystyrene	1000	2536	< 0.001	2348	< 0.001	92	0.412	467	< 0.001	2014	0.054	54	< 0.001

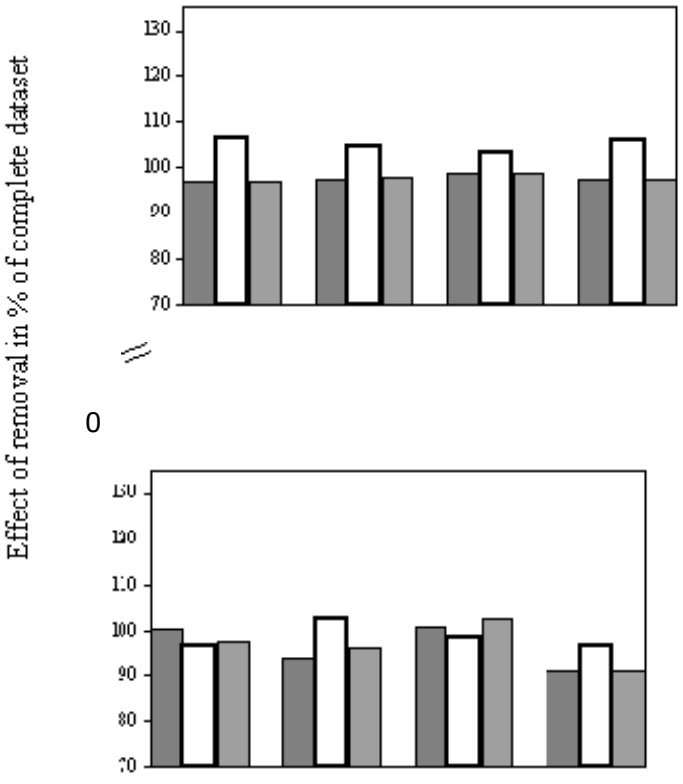
Fig.1. Regression of *Rotylenchulus reniformis* or *Meloidogyne incognita* nematode count (vermiform or J2, respectively), egg count, and reproductive factor on soil volume for PM 1218 inoculated at 7 d with 1000 vermiform (J2) life stages and eggs per pot. Regression coefficients are given in Table 2.





Pot material means

Standard error of the mean



Pot material differences

Standard error of the difference

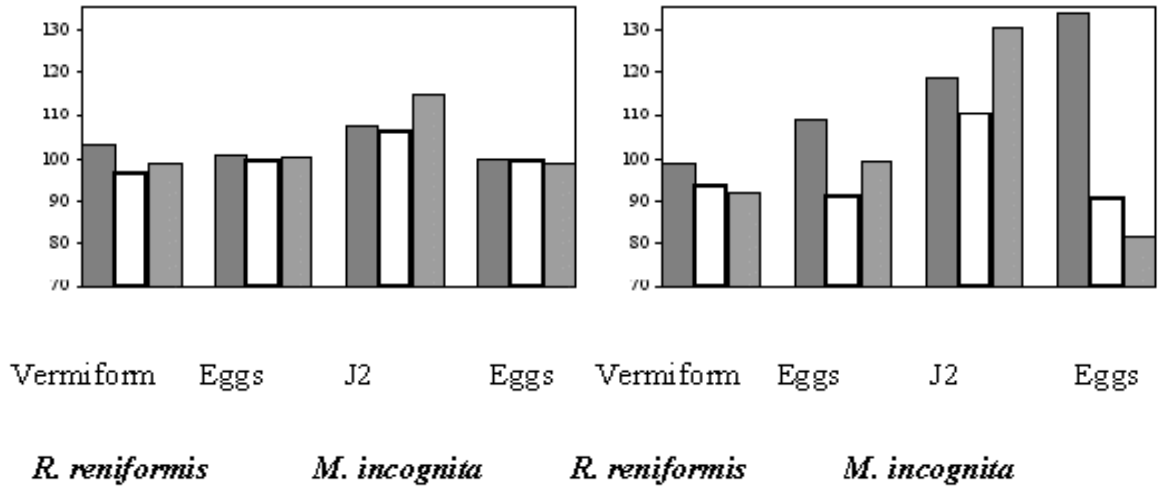


Fig. 2. Effect of removal of replicate with the highest count, lowest count or highest and lowest count (vermiform, J2, eggs) on the relative magnitude of the reproductive factor (Rf) compared to 10 replicates within a run of the experiment.

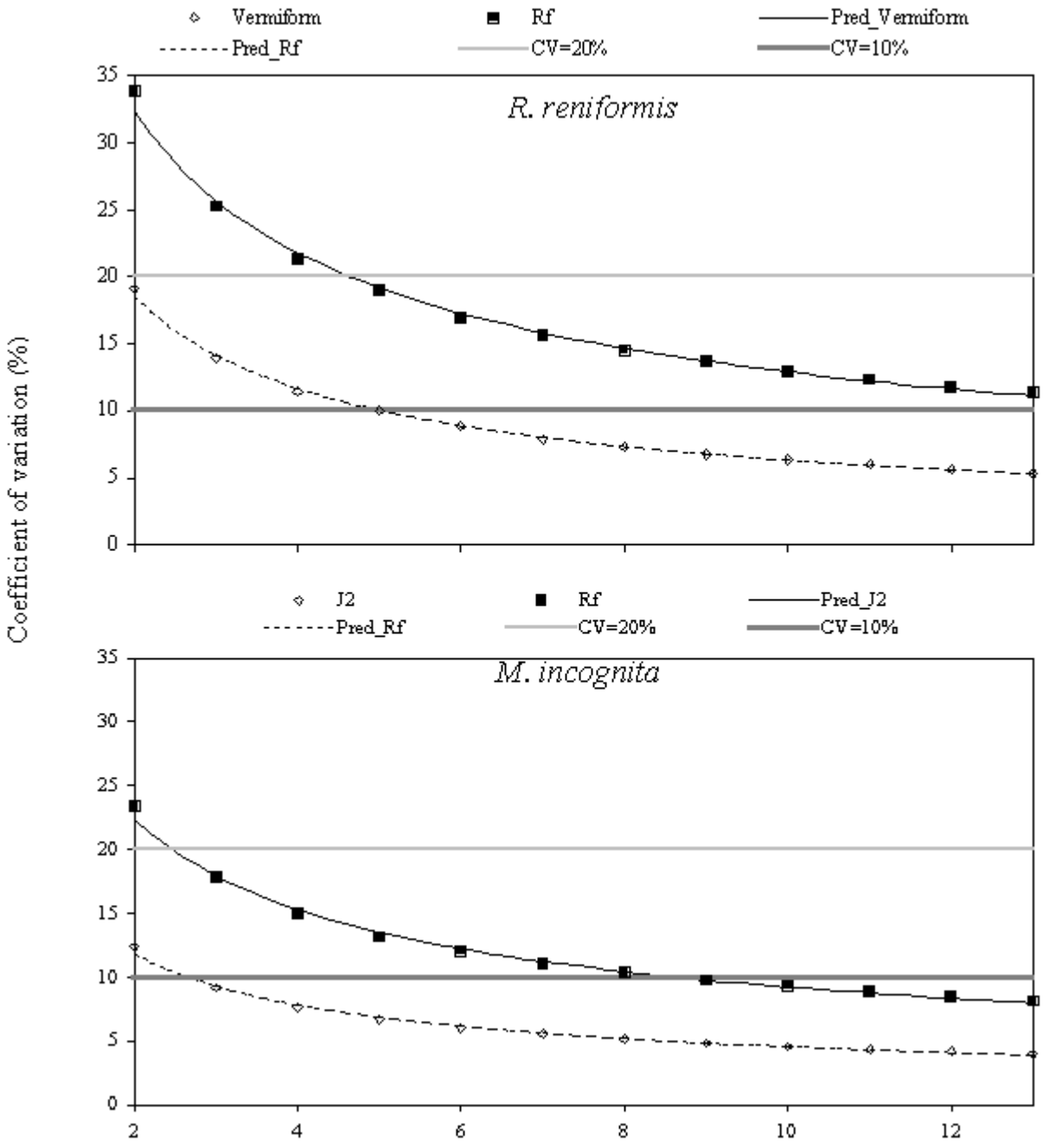


Fig. 3. Relationship between number of replicates and coefficient of variation (CV = 100 * STDERR/MEAN) for *R. reniformis* (vermiform, Rf) and *M. incognita* (J2, Rf) based on sampling all 20 replicates from two complete experimental runs for each species 1000 times. Data for each sampling x sample size combination were analyzed by Proc Mixed and the CV calculated. Data points given correspond to the 90th percentile of all CVs from 1000 sampling events. The horizontal lines represent CV benchmarks of 20 and 10%.