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Probability of detecting nematode infestations for quarantine sampling with imperfect extraction efficacy

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Abstract: For quarantine sampling, it is of fundamental importance to determine the probability of finding an infestation when a specified number of units are inspected. In general, current sampling procedures assume 100% probability (perfect) of detecting a pest if it is present within a unit. Ideally, a nematode extraction method should remove all stages of all species with 100% efficiency regardless of season, temperature, or other environmental conditions; in practice however, no method approaches these criteria. In this study we determined the probability of detecting nematode infestations for quarantine sampling with imperfect extraction efficacy. Also, the required sample and the risk involved in detecting nematode infestations with imperfect extraction efficacy are presented. Moreover, we developed a computer program to calculate confidence levels for different scenarios with varying proportions of infestation and efficacy of detection. In addition, a case study, presenting the extraction efficacy of the modified Baermann's Funnel method on *Aphelenchoides besseyi*, is used to exemplify the use of our program to calculate the probability of detecting nematode infestations in quarantine sampling with imperfect extraction efficacy. The result has important implications for quarantine programs and highlights the need for a very large number of samples if perfect extraction efficacy is not achieved in such programs. We believe that the results of the study will be useful for the determination of realistic goals in the implementation of quarantine sampling.

Key words: Quarantine sampling, detecting nematode infestations, modified Baermann's Funnel method, binomial distribution, hypergeometric distribution, Monte Carlo simulation method.

Quarantine refers to regulatory actions aimed at preventing or retarding the introduction, establishment and spread of dangerous pests in crop protection (Maas, 1987). In quarantine and certification programs, intensive sampling may be needed to determine if lots or shipments of plants, pots, cuttings, or other units are free of plant-parasitic nematodes and other plant pests (McSorley and Littell, 1993). Because it is seldom feasible, or even possible, to examine and test entire lots for such harmful pests, these determinations must nearly always be made on the basis of samples drawn from the lots.

Sometimes a low level of disease intensity carries a disproportionately high risk of introducing the pests (Madden et al., 2007), and some diseases are so destructive as to warrant strict regulatory control (Clayton and Slack, 1988). A previous study applied a three-step procedure to rank exotic pests according to their expected economic impact (EEI) in the USA (Maas, 1987). Among the 49 top-ranking exotic pests, several EEIs are above US $300 million each. Because, in the case of quarantine pests, the introduction of even a few injurious organisms can lead to disastrous consequences, zero acceptance number sampling plans are often adopted. In fact, a zero acceptance number within a sample does not imply a zero tolerance level in the entire lot. Even if no pests are detected in the sample, there remains a probability that the pest may be present in the rest of the lot.

A probabilistic statement is possible in order to address the aforementioned problem (McArdle, 1990). Venette et al. (2002) discussed the idea that inspection using statistically based sampling methods can provide results with a certain level of confidence. Moreover, FAO (2008) discussed the idea that the sampling methodologies in selecting samples for the inspection in the entire lot be based on a number of parameters such as acceptance level, proportion of infestation, confidence level, efficacy of detection, and sample size.

In the Nematology literature, McSorley and Littell (1993) provided a methodology for determining confidence levels for various proportions of infestation, lot sizes, and sample sizes in zero acceptance number sampling plans. The confidence level indicates the probability of detecting at least one infested unit in a lot. For example, a 95% confidence level means that the conclusions drawn from the results of sampling will detect a noncompliant lot, on average, 95 times out of 100, and therefore, it may be assumed that, on average, 5% of non-compliant lots will not be detected.

One of the assumptions in the work of McSorley and Littell (1993) is that there is 100% probability (perfect) of detecting a pest within a unit that is infested in quarantine sampling. In practice however, detection often doesn't approach 100% efficiency, especially in soil sampling. In general, methods applied to separate plant parasitic nematodes from the embedding substance are based on differences in body size, density, or mobility. These include Baermann's funnel method, sugar floatation and sieving methods (Viglierchio and Schmitt, 1983; McSorley and Parrado, 1987; Robinson and Heald, 1989). These methods often have only 30-80% efficiency, and some research results even indicate that these methods are all less than 50% efficient (McSorley and Littell, 1993; Viglierchio and Schmitt, 1983). Ideally, a nematode extraction method should

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remove all stages of all species with 100% efficiency regardless of season, temperature, or other environmental conditions, but no currently used method approaches these criteria (McSorley, 1987).

When the extraction efficacy is assumed to be not perfect, the confidence level is lower than it would be under the assumption of 100% detection of an infection within a unit. Within a lot size, the confidence level increases as either the proportion of infestation or the number of collected samples is increased (McSorley and Littell, 1993). Thus, to keep the same confidence level, it is necessary to increase the numbers of samples for the fixed proportion of infestation. Nevertheless, little investigation has centered on the quantitative aspects of such situations for quarantine sampling. For example, how large a sample size is enough to compensate for the imperfect extraction efficacy? How large will the risk be if we ignore the fact that the extraction efficacy is imperfect?

In this paper, we mainly focus the discussion on extraction efficacy because it often doesn't approach 100%. The result has important implications for quarantine programs and highlights the need for a very large number of samples if perfect extraction efficacy is not achieved in such programs. Although a previous report (FAO, 2008) was presented to account for the consideration of imperfect efficacy of detection, it treated the efficacy of detection as a fixed value. In some situations, the extraction efficacy and the proportion of infestation will probably vary within ranges which can be determined by results obtained from a single extraction method run at various times. For example, the extraction efficacy is less than the specified value, or the proportion of infestation is known to be greater than one value and less than another value. McSorley (1987) listed a wide range of extraction efficiencies, which may be fixed or may vary depending on soil type, method used, and the target nematode species, etc. Thus, in this paper we deal with four scenarios for the efficacy of detection or the proportion of infestation to meet the actual requirements of particular conditions (see Appendix).

Because the efficacy of detection and the proportion of infestation may vary, it is not appropriate to select only one of many possible values for the uncertain parameters. To solve this problem, we employed Monte Carlo simulation method, using different combinations of the uncertain parameters as inputs. The degree of variation between the outputs of different simulations will reveal the level of certainty for any particular estimate (e.g., mean, median, etc.) of the parameters of interest (Brattin et al., 1996).

In a relevant study of plant protection, Clayton and Slack (1988) discussed the sampling schemes in zero tolerance regimes for bacterial ring rot in potatoes. They presented the concept of the probability of erroneously accepting (PEA), in which disease present in the field will be missed in the sample. In fact, PEA is the complement of the confidence level discussed in this paper. For example, if PEA equals 5%, it means that there is a 5% chance that disease present will be missed. Conversely, there is a 95% chance of detecting at least one infested unit. Therefore, we will use the concept of PEA to assess the risk whenever we ignore the fact that that the extraction efficacy is imperfect.

The objective of this study is to determine the probability of detecting nematode infestations for quarantine sampling when extraction efficacy is imperfect. Moreover, a computer program has been developed to calculate confidence levels for the different scenarios for the proportion of infestation and the efficacy of detection. A case study, presenting the extraction efficacy of the modified Baermann's Funnel method on *Aphelenchoides besseyi*, is used to exemplify the use of the program to calculate the probability of detecting nematode infestations in quarantine sampling when the extraction efficacy is not perfect. We believe that the results of the study will be useful for the determination of realistic goals in the implementation of quarantine sampling.

Materials and Methods

Extraction efficacy of the modified Baermann's Funnel method: Baermann's Funnel method has been widely used for its low set up input and it's convenience of operation. A critical analysis of different extraction methods was made and concluded that the Baerman method was the most efficient and that there was no significant difference between the number of nematodes extracted by the sedimentation and sieving techniques (Harrison and Green, 1976). Thus, the extraction efficacy of this method was tested in this study. *Aphelenchoides besseyi* was selected for the default test because of its notoriety as a quarantine nematode in many countries. Here, to demonstrate the developed computer program, we used the extraction efficacy of the modified Baermann's Funnel method (Wu et al., 2010), which obtains nematodes by artificial seeding of soil samples with known numbers of nematodes.

The funnel used in this study was open ended made of plastic and was 14 cm in diameter. For nematode collection, a small glass vial (1.4 cm diameter) was attached to the funnel with a rubber tube (8 cm long). Two tissue papers (Kimberly-Clark ®, Taipei, Taiwan) were placed on a mesh, and 100 g sand, with particle diameters between 0.42-0.84 mm, was placed on the tissue. One thousand all-stage *Aphelenchoide besseyi* were added evenly into the sand, and the mesh containing the sand and nematodes was placed on top of the funnel. Water was immediately added nearly to the brim of the funnel to cover the sample; no mist was applied during the incubation period. Nematodes from each funnel were collected twice at 24-hr and 48-hr intervals. In the case study, three replicates were used in the experiment.

The entire experiment was repeated five times to determine the repeatability of treatment in the laboratory. We also investigated whether the extraction efficacies were consistent across experiments.

Statistical models: In general, in order to characterize units as infested or not, the probability of any combination of infested and non-infested units in a sample can be determined from the binomial or from the hypergeometric distribution, when the distribution of the pest among units is random and the sampling of units is random.

For large lots sufficiently mixed, the likelihood of finding an infested unit is approximated by binomial distribution. The sample size is less than 5% of the lot size. Binomial sampling is based on sampling with replacement. The probability of observing i infested units in a sample of n units is given by:

$$\mathrm{P}_r(x = i) = \binom{n}{i} p^i (1-p)^{n-i}, \tag{1}$$

where p is the proportion of infested items.

The hypergeometric distribution is appropriate for describing the probability of finding a pest in a relatively small lot. A lot is considered to be small when the sample size is more than 5% of the lot size. In this case, sampling of one unit from the lot affects the probability of finding an infested unit in the next unit selected. Hypergeometric-based sampling is based on sampling without replacement. This probability is given by

$$\mathrm{P}_r(x = j) = \frac{\binom{K}{j}\binom{L-K}{n-j}}{\binom{L}{n}}, \tag{2}$$

where K is the number of infested units in the lot, j is the number of infested units in the sample, L is the total number of units in the lot, and n is the number of units in the sample (Venette et al., 2002).

The methodology for extracting the nematodes often cannot achieve 100% extraction efficacy; thus the results will involve one or more assay errors. Generally, there are two types of assay errors (Cowling et al., 1999; Williams & Moffitt, 2010). First, there is the probability of falsely detecting defective items when defective items do not exist in reality (denoted by δ). Secondly, there is the probability of failing to detect defective items when in reality defective items exist (denoted by λ). $1-\lambda$ represents the extraction efficacy in the Nematology case study. The first type of error is ignored because the confirmations of the extracting results are visual observations. The specific plant parasitic nematodes to be isolated for research or quarantine purposes usually have distinct morphological characteristics. A well-trained nematologist or inspector will not misidentify the target nematode; therefore the impact of specificity ($1-\delta$) will not be addressed here. So, in this study we determined the probability of detecting at least one infested unit with imperfect extraction efficacy for quarantine sampling.

Based on the above assumption, the true infestation rate of units should be adjusted to $p_a = (1-\lambda)p$, where p is the true infestation rate and p_a is the adjusted infestation rate determined by the assay method. It is easy to show that the adjusted infestation rate (p_a) decreases as the extraction efficacy $(1-\lambda)$ decreases. Therefore, the binomial distribution probability of detecting zero infested units in sample size (n) when taking the extraction efficacy $(1-\lambda)$ into account is:

$$(1-p_a)^n = [1-(1-\lambda)p]^n \tag{3}$$

Also, the probability of detecting at least one infested unit can be written as

$$1-(1-p_a)^n = 1-[1-(1-\lambda)p]^n \tag{4}$$

For the hypergeometric distribution, the probability of detecting zero infested units in sample size (n) from a lot of L units adjusted for imperfect extraction efficacy, which contains K_1 infested units, is as follows:

$$\frac{(L-K_1)\,!\,(L-n)\,!}{L\,!\,(L-K_1-n)\,!} \tag{5}$$

where $K_1 = L \times p_a = L \times (1-\lambda) \times p$, which is the smallest integer greater than $L \times p_a$. Hence, the probability of detecting at least one infested unit using the hypergeometric distribution is as follows:

$$1-\frac{(L-K_1)\,!\,(L-n)\,!}{L\,!\,(L-K_1-n)\,!}. \tag{6}$$

Expressions (3) and (5) are the probabilities of erroneously accepting (PEA) to assess the performance of the different sampling schemes on binomial and hypergeometric probability distributions, respectively.

Monte Carlo simulation method: When values for the extraction efficacy and the true infestation rate are both fixed, expression (4) or (6) is employed to calculate the probability of detecting at least one infested unit. However, when these values are not fixed, it is necessary to determine ranges of variation based on real world conditions. Then, using these ranges, a Monte Carlo simulation is performed to account for the uncertainty of the parameters of interest. A Monte Carlo simulation is a technique that involves using random sampling and probability to solve problems (Metropolis and Ulam, 1949). Since this method is based on repeated computation of random numbers, calculation by computers is required and this method tends to be used when it is unfeasible or impossible to compute an exact result with a deterministic algorithm. In this study, the adjusted infestation rate of the assay method is expressed as a product of the extraction efficacy and the true infestation rate. To take into account the uncertainty both

in the extraction efficacy and in the true infestation rate, both of which affect the adjusted infestation rate, the extraction efficacy and the true infestation rate need to be specified separately for each of the particular distributions (Rai and Krewski, 1998).

A beta distribution (*Beta* (a, b)), defining the distribution of a random variable on the closed unit interval[0, 1], can be made very flexible by choosing different shape parameters a and b based on expert knowledge or previous data (Cowling et al., 1999; Williams and Moffitt, 2010). Thus, it would be a logical choice for defining a distribution of the values of the extraction efficacy and the true infestation rate. Traditionally, if r individuals are positive among n examined, then the parameters of the distribution can be calculated as $a = r + 1$ and $b = n - r + 1$. These parameters can be specified by provided estimates of the mode and 5% or 95% confidence limits, both available from expert opinion or previous data. Previous papers (Branscum et al., 2005; Messam et al., 2008) have presented some useful software to deal with the situation. Their free software called "BetaBuster" is available at <http://www.epi.ucdavis.edu /diagnostictests/betabuster.html> and can be used to determine the parameters of specific beta prior distributions based on scientific input. For example, if a laboratory assay assumes 95% confidence that the extraction efficacy is less than 50% and that the accuracy concentrates around 30%, "Betabuster" will take the information and obtain the unique Beta distribution with mode at 30%, and with 95% of the area of the distribution to the left side of 50%. For the above case, *Beta* (6.2809, 13.3221) will be specified. In this study, we obtained information about the extraction efficacy by the experimental results of the modified Baermann's Funnel method for *Aphelenchoides besseyi*.

To account for uncertainty using Monte Carlo simulation, we followed the steps listed below:

Step 1: Based on the specified beta distributions, generate 50,000 sets of random samples each for the extraction efficacy and for the infestation rate.
Step 2: Using the results of Step 1, calculate the binomial or hypergeometric distribution probability of detecting at least one infested unit in a sample lot by expressions (4) or (6).
Step 3: Calculate the medians of the outputs of Step 2.
Step 4: Repeat Steps 1, 2, and 3 for 100 times and calculate the mean of the resulting medians.

We provide a computer program (see Appendix) based on Monte Carlo simulation, performed in a statistical software R to obtain the probabilities of detecting at least one infestation unit as a function of four factors: lot size, number of samples, level of infestation, and extraction efficacy. R software available at <http://www.r-project.org/> is a free software environment that includes a set of base packages for graphics, math, and statistics. There are some useful books which introduce R programming environment (Dalgaard, 2002; Venables and Smith, 2002).

Results

Characteristics of the probabilities of detecting at least one infested unit: First, we investigated the relationships among varying proportions of infestation, efficacy of detection, sample size, and confidence level (the probability of detecting at least one infested unit) in zero acceptance number sampling plans. Fig. 1 shows the relationships of the confidence levels to a range of sample sizes for the different infestation rates (p) and the extraction efficacy ($1 - \lambda$). The confidence level increases as the infestation rate and the sample size increase. For a fixed sample size, the confidence level decreases as the extraction efficacy decreases, regardless of the infestation rate.

Secondly, the probabilities were calculated using the binomial distribution, to achieve a 95% confidence level for detecting at least one infested unit. The relationship of the sample size to a range of infestation rates for different levels of extraction efficacy is shown in Fig. 2. For a fixed value of the infestation rate, the sample size increases as the extraction efficacy decreases.

Probability of Erroneously Accepting (PEA): If the proportion of infestation is 0.01, the probability that it will wrongly be concluded, on the basis of sampling, that a lot is pest-free is at most 0.05. Then using expression (3) and assuming perfect extraction efficacy, the sample size equals 299 ($n = \log(0.05)/\log(1 - 0.01)$)(rounding to an integer value). Therefore a sample size of 299 is required in order to be able to make the statement that there is a 0.05 risk that the actual proportion of infestation exceeds 0.01. As used here, a PEA is synonymous with a risk, carrying the connotation that the probability in question is that of an undesirable outcome.

When adjusting for the extraction efficacy, the adjusted infestation rate is lower than the actual infestation rate; therefore the PEA will be changed. For example, following the aforementioned case, when the extraction efficacies are 0.7, 0.5, and 0.2, the adjusted infestation rates will be changed to 0.007, 0.005, and 0.002, respectively. Keeping the same sample size "299", the corresponding risks, by expression (3), are 12%, 22%, and 55%, respectively. Thus, if we ignore the fact that the extraction efficacy is imperfect, the sample size in the situation of perfect extraction efficacy is used and this leads to a great risk.

Case study-extraction efficacy of Aphelenchoides besseyi using the modified Baermann Funnel method: The extraction efficacy for *Aphelenchoides besseyi* using the modified Baermann Funnel method varied from 14.9% to 43.4%. The mean and mode of the extraction efficacies from 15 data were 25.5% and 24.8%, respectively (Table 1).

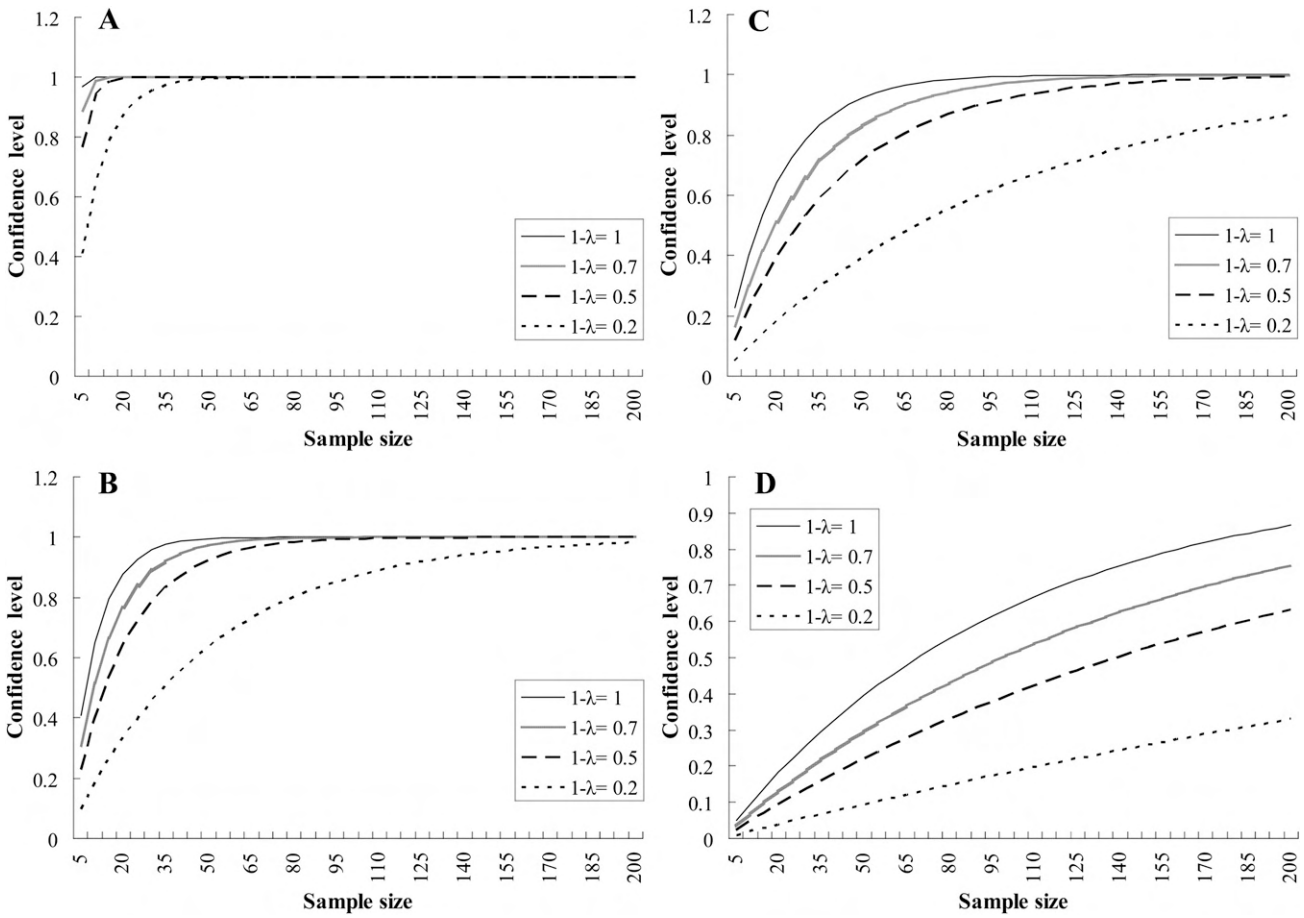

FIG. 1. Relationships of confidence levels to a range of the sample sizes for different infestation rates (p) and extraction efficacies ($1-\lambda$). A) $p = 0.5$. B) $p = 0.1$. C) $p = 0.05$. D) $p = 0.01$.

The data were analyzed using PROC GLM in SAS (Cary, NC, version 9.2). The calculated F value was compared with the tabular F value for 4 and 10 df to decide whether to accept the null hypothesis of no difference between population means across experiments. The calculated F value for 4 and 10 df was 1.18 and its P-value was 0.378 (that is, $P \geq 0.05$). Thus, we concluded that the extraction efficacies for the case study were consistent across experiments.

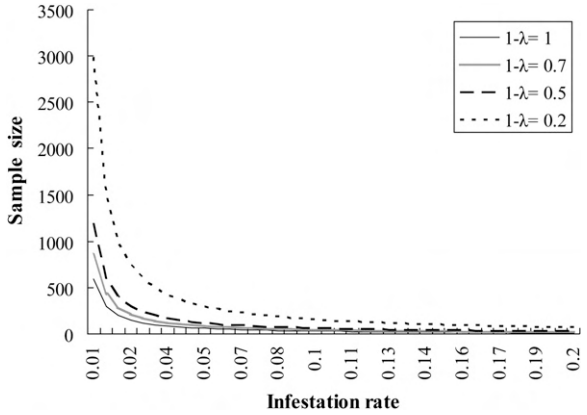

FIG. 2. To achieve a 95% confidence level, the required relationships of the sample size to a range of infestation rates for different levels of extraction efficacy ($1-\lambda$).

Probabilities of detecting target pests under different sampling numbers and infestation rates for a range of lot sizes: To obtain the probability of detecting infestations in different lot sizes and numbers of samples with imperfect extraction efficacy from the Monte Carlo simulation, first we should specify the parameters of the beta distributions of the extraction efficacy, and of the infestation rate. Here, the beta distribution of the extraction efficacy for extracting the nematodes is based on the previous experimental result. Therefore, the extraction efficacy is assumed to be the beta distribution with mode at 24.8% and the 99th percentile at 43.4% (the maximum value of the experiment). *Beta*(10.5016, 29.8114) was specified by the "Betabuster" software. For the infestation rate, the beta distribution can be specified by expert opinion. In the case of an infestation rate equal to or less than 5%, the beta distribution with mode at 1% and the 95th percentile at 5% was specified as *Beta*(1.8816, 88.2800).Similarly, the distribution *Beta*(5.6192, 42.5732) was specified for the case of the infestation rate equal to or less than 20% when the mode and the 95th percentile of this specified beta distribution were at 10% and 20%, respectively.

TABLE 1. The extraction efficacy for 1000 *Aphelenchoides besseyi* using the modified Baermann Funnel method for 48 hrs.

Treatment		Experiment 1			Experiment 2			Experiment 3			Experiment 4			Experiment 5		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Aphelenchoides besseyi	1,000 nematodes per 100g sand[a]	24.8%	25.3%	22.1%	21.4%	24.7%	43.4%	27.5%	27.3%	36.0%	26.2%	14.9%	19.7%	22.5%	30.1%	17.7%

[a] The diameters of sand particles were between 0.42 mm and 0.84 mm.

Secondly, taking the uncertainties of the extraction efficacy and infestation rate into account, Table 2 presents the probabilities of detecting at least one infested units, which were averaged over 100 simulated random samples each with 50,000 times for a range of lot sizes and numbers of samples with imperfect extraction efficacy when the infestation rate was fixed or varied within a pre-determined range.

Table 2 shows that when the probabilities were calculated using the binomial distribution, more than 200 samples were needed to detect target nematodes at a 95% confidence level when the infestation rate was at 5% using the modified Baermann's Funnel method. If the infestation rate was at 1% in the lot, more than 1,000 samples were needed to catch the target pests (Table 2). For the probabilities calculated using the hypergeometric distribution, since the infested units must be taken as the smallest integer greater than $L \times p_a$, the probability of detecting at least one infested unit is equal to or larger than that of the binomial distribution for any situation.

The comparison of "10%" and "≤ 20% with the mode at 10%" for the infestation rates is interesting (Table 2). The result of the former is always less than that of the latter. This means that if the data information varies, we should use the information to obtain a higher confidence level. In the appendix, we show the computer program in detail to account for the four scenarios, the extraction efficiency or the infestation rate being fixed or varying within set limits. Also, the program takes into account whether the lot size is known or unknown. Thus, the situation of 100% extraction efficacy is also included as a special case.

DISCUSSION

Our paper has focused on the probability of detecting nematode infestations for quarantine sampling with imperfect extraction efficacy. We have shown the relationships among proportion of infestation, confidence level, efficacy of detection, and sample size, in zero acceptance number sampling plans. Moreover, we developed a computer program to calculate the confidence levels of the different scenarios for the proportion of infestation and the efficacy of detection. Also, a case study was used to exemplify the use of the

TABLE 2. Probability[a] of detecting at least one infested unit in different lot sizes, numbers of samples, and infestation rates, with imperfect extraction efficacy assay, distributed from the beta distribution with mode at 24.8% and 99th percentile at 43.4% from Table 1.

Lot size (L)	Number of samples (n)	Infestation rate (p)					
		50% infested	10% infested	5% infested	1% infested	≤5%[b]	≤20%[c]
100	5	0.4966 (0.5092)	0.1219 (0.1440)	0.0625 (0.0980)	0.0128 (0.0500)	0.0215 (0.0500)	0.1317 (0.1440)
100	10	0.7465 (0.7689)	0.2288 (0.2735)	0.1211 (0.1909)	0.0254 (0.1000)	0.0426 (0.1000)	0.2461 (0.2735)
100	20	0.9358 (0.9557)	0.4054 (0.4919)	0.2275 (0.3616)	0.0501 (0.2000)	0.0834 (0.2000)	0.4317 (0.4919)
100	30	0.9837 (0.9933)	0.5415 (0.6615)	0.3210 (0.5121)	0.0742 (0.3000)	0.1224 (0.3000)	0.5713 (0.6615)
100	50	0.9990 (>0.9999)	0.7272 (0.8788)	0.4756 (0.7525)	0.1205 (0.5000)	0.1957 (0.5000)	0.7565 (0.8788)
1000	10	0.7465 (0.7500)	0.2288 (0.2325)	0.1211 (0.1232)	0.0254 (0.0297)	0.0426 (0.0491)	0.2461 (0.2483)
1000	20	0.9358 (0.9385)	0.4054 (0.4126)	0.2275 (0.2322)	0.0501 (0.0589)	0.0834 (0.0963)	0.4317 (0.4367)
1000	50	0.9990 (0.9992)	0.7272 (0.7410)	0.4756 (0.4888)	0.1205 (0.1428)	0.1957 (0.2266)	0.7565 (0.7672)
1000	100	>0.9999 (>0.9999)	0.9256 (0.9377)	0.7250 (0.7480)	0.2265 (0.2712)	0.3532 (0.4102)	0.9407 (0.9502)
1000	200	>0.9999 (>0.9999)	0.9945 (0.9972)	**0.9244** (0.9461)	0.4018 (0.4884)	0.5813 (0.6731)	0.9965 (0.9982)
1000	300	>0.9999 (>0.9999)	0.9996 (>0.9999)	0.9792 (0.9906)	0.5371 (0.6574)	0.7289 (0.8347)	0.9998 (>0.9999)
1000	500	>0.9999 (>0.9999)	>0.9999 (>0.9999)	0.9984 (0.9999)	0.7231 (0.8754)	0.8868 (0.9691)	>0.9999 (>0.9999)
10000	10	0.7465 (0.7469)	0.2288 (0.2293)	0.1211 (0.1217)	0.0254 (0.0257)	0.0426 (0.0432)	0.2461 (0.2467)
10000	50	0.9990 (0.9990)	0.7272 (0.7288)	0.4756 (0.4782)	0.1205 (0.1223)	0.1957 (0.1983)	0.7565 (0.7580)
10000	100	>0.9999 (>0.9999)	0.9256 (0.9270)	0.7250 (0.7285)	0.2265 (0.2302)	0.3532 (0.3580)	0.9407 (0.9420)
10000	500	>0.9999 (>0.9999)	>0.9999 (>0.9999)	0.9984 (0.9987)	0.7231 (0.7369)	0.8868 (0.8959)	>0.9999 (>0.9999)
10000	1000	>0.9999 (>0.9999)	>0.9999 (>0.9999)	>0.9999 (>0.9999)	**0.9233** (0.9356)	0.9872 (0.9904)	>0.9999 (>0.9999)
100000	100	>0.9999 (>0.9999)	0.9256 (0.9258)	0.7250 (0.7254)	0.2265 (0.2270)	0.3532 (0.3537)	0.9407 (0.9409)
100000	1000	>0.9999 (>0.9999)	>0.9999 (>0.9999)	>0.9999 (>0.9999)	0.9233 (0.9247)	0.9872 (0.9875)	>0.9999 (>0.9999)

[a] The probabilities were calculated using the binomial distribution and hypergeometric distribution (in parentheses).
[b] The infestation rate is distributed from the beta distribution with the mode at 1% and the 95th percentile at 5%.
[c] The infestation rate is distributed from the beta distribution with the mode at 10% and the 95th percentile at 20%.

program to calculate the probability of detecting nematode infestations in quarantine sampling with imperfect extraction efficacy. From the case study, we perceive that if the data information varies, we should use the information about the range of variation to obtain the higher confidence level instead of using the point estimate. When considering the concept of PEA and using the sample size in the situation of perfect extraction efficacy, a risk, a probability of misclassification of unacceptable lots as acceptable, increases drastically if we ignore the fact that the extraction efficacy is imperfect. In addition, for the case study, the required sample size with imperfect extraction efficacy is much larger than the required sample size when assuming perfect extraction efficacy. The main reason for the dramatic increase in required sample size is the low extraction efficacy.

The confidence level when the extraction efficacy is not perfect can be lower than the confidence level when under the assumption of 100% detection of an infection within a unit. Thus, to keep the same confidence level, it is necessary to increase numbers of samples for the fixed proportion of infestation. However, it is sometimes impractical to increase a sampling number to 1,000 or to a larger amount in order to accommodate the imperfect extraction efficacy of the current methods provided. In order to lower the risk of introducing the pathogens, increasing the sensitivity of the identification protocol is one of the possible methods. Molecular biology tools have generated much useful information for nematode diagnosis (Powers, 2004), and should provide a more accurate and economical method for detecting the zero tolerance pests under quarantine regulation.

Systems approaches may be alternative measures which can address the problem in which the sample size increases drastically when the extraction efficacy is not perfect. A systems approach requires the integration of different measures, at least two of which act independently, with a cumulative effect. Those measures can be applied in the place of production, during the post harvest period, at the packinghouse, or during shipment and distribution of the commodity. An advantage of the systems approach is the ability to address variability and uncertainty by modifying the number and strength of measures in order to meet the appropriate level of phytosanitary protection and confidence (FAO, 2002).

When plant pathogens are introduced into an area in which host plants have been growing in the absence of the pathogen, such introduced pathogens may cause much more catastrophic epidemics than the existing endemic pathogens (Agrios, 2005). For some quarantine regulated nematode species, such as *Globodera rostochiensis* and *G. pallida*, the identification of a single individual can lead to the rejection of an entire shipment of potatoes intended for trading. Thus, zero acceptance number sampling plans are often adopted in quarantine regulations to prevent disastrous consequences.

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Appendix

For the method proposed in this paper, we have provided R function to calculate the binomial and hypergeometric distribution probabilities of detecting nematode infestations in quarantine. To take into account the uncertainties of extraction efficacy and infestation rate, four scenarios are considered:

I. The extraction efficacy and the infestation rate are fixed.
II. The extraction efficacy varies within a pre-determined range; the infestation rate is fixed.
III. The extraction efficacy is fixed; the infestation rate varies within a pre-determined range.
IV. The extraction efficacy and the infestation rate vary within pre-determined ranges.

In addition, the program takes into account whether the lot size is known or unknown. In this R program, "p" and "Se" are the specified values for the infestation rate and the extraction efficacy, respectively. (pa, pb) and (Sea, Seb) obtained by "Betabuster" are the Beta distribution parameters of the infestation rate and the extraction efficacy, respectively. For example, in the case of the infestation rate being equal to or less than 5%, the beta distribution with the mode at 1% and the 95th percentile at 5% was specified as *Beta*(1.8816, 88.2800). Thus, (pa, pb) is set to be (1.8816, 88.2800).Also, "L" and "n" are denoted by the lot size and the number of samples, respectively. The R program used is as follows:

```
infestation=function(p,pa,pb,Se,Sea,Seb,n,L){
q50=hq50=rep(0,100)
for (i in 1:100){
if (is.na(p)=="FALSE" && is.na(Se)=="FALSE"){
plow=p
λ=Se}
if (is.na(p)=="FALSE" && is.na(Se)=="TRUE"){
plow=p
λ=rbeta(50000,Sea,Seb)}
if (is.na(p)=="TRUE" && is.na(Se)=="FALSE"){
plow=rbeta(50000,pa,pb)
λ=Se}
if (is.na(p)=="TRUE" && is.na(Se)=="TRUE"){
plow=rbeta(50000,pa,pb)
λ=rbeta(50000,Sea,Seb)
}

pt=λ*plow
PH=1-(1-pt)^n
if (is.na(L)=="TRUE"){
HP=rep(0,50000)}
else {HP=1-dhyper(0,n,L-n,ceiling(pt*L))}

q50[i]=round(quantile(PH,0.5),5)
hq50[i]=round(quantile(HP,0.5),5)
}
q50=round(mean(q50),5)
hq50=round(mean(hq50),5)

if (is.na(L)=="TRUE"){list("Binomial distribution"=q50)}
else {list("Binomial distribution"=q50,"Hypergeometric distribution"=hq50)}
}
```

Some examples are presented. First, assume "n" and "L" are 10 and 100, "p" and "Se" are specified as 0.1 and 0.2, which is the case of fixed values for the infestation rate and the extraction efficacy. Then the probabilities of detecting infestations can be obtained by inputting the following commands.

```
> infestation(p = 0.1, pa = NA, pb = NA,
Se = 0.2, Sea = NA, Seb = NA, n = 10, L = 100)
$'Binomial distribution'
[1] 0.18293

$'Hypergeometric distribution'
[1] 0.27347
```

Secondly, as in the case study in the paper, assume that the distribution of the extraction efficacy is specified as *Beta*(10.5016, 29.8114)obtained by "Betabuster". That is, (Sea, Seb) are (10.5016, 29.8114), and the values for "p", "n" and "L" are 0.1, 10 and 100, respectively. For this case, we can enter the following R codes.

```
> infestation(p = 0.1, pa = NA, pb = NA,
Se = NA, Sea = 10.5016, Seb = 29.8114,
n = 10, L = 100)
$'Binomial distribution'
[1] 0.22881

$'Hypergeometric distribution'
[1] 0.27347
```

Thirdly, if the mode and the 95th percentile of the infestation rate are 10% and 20%, the infestation rate is

specified as *Beta*(5.6192, 42.5732),and the values of "Se", "n" and "L" are 0.2, 10 and 100. Thus, (pa, pb) are specified as (5.6192, 42.5732); we can enter the following R codes.

```
> infestation (p = NA, pa = 5.6192,
pb = 42.5732, Se = 0.2, Sea = NA, Seb = NA,
n = 10, L = 100)
$'Binomial distribution'
[1] 0.20149

$'Hypergeometric distribution'
[1] 0.27347
```

Fourthly, assume "n" and "L" are 10 and 100, and the infestation rate & the extraction efficacy vary within ranges specified as *Beta*(5.6192, 42.5732) and *Beta*(10.5016, 29.8114), respectively. The probabilities of detecting infestations can be obtained by the following commands.

```
> infestation (p = NA, pa = 5.6192,
pb = 42.5732,Se = NA, Sea = 10.5016,
Seb = 29.8114, n = 10, L = 100)
$'Binomial distribution'
[1] 0.24609

$'Hypergeometric distribution'
[1] 0.27347
```

In addition, if we have no idea of the value of the lot size "L", this R function can only provide the binomial distribution probability of detecting infestations. The result can be obtained by the following commands.

```
> infestation (p = 0.1, pa = NA, pb = NA,
Se = 0.2, Sea = NA, Seb = NA, n = 10, L = NA)
$'Binomial distribution'
[1] 0.18293
```