Estimation of nematode infestation in banana.

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INTRODUCTION

Methods and techniques used for assessment of nematode infestations are quite varied and the choice of which one of them to use will depend on many factors such as purpose of the study and accuracy needed, cropping system, nematode species and, technical (equipment) limitations (see Southey, 1985 ; Hooper, 1985 a and 1985 b ; Ferris, 1987 ; Seinhorst, 1988). This consideration is directly applicable to the multifaced system of banana production and estimation methods will range from direct evaluation in the field through damage assessment up to sophisticated methods of sampling and extraction.

DIRECT ESTIMATION IN THE FIELD

Nematode damage on banana can be directly assessed in the field by determining the extent of necroses on corm or roots (migratory), or gall index on roots (sedentary). A 0 to 4 scale was developed to assess the extent of necroses on banana roots (Stover, 1972). Necroses are due to both nematodes and pathogens (mainly fungi). One of these fungi, *Cylindrocladium*, is pathogenic in the absence of nematodes (Loridat and Ganry, 1989). However, the relationship between tissue necroses or galls and endoparasitic nematode infestation is usually consistent in the field.

Twice-monthly counts of uprooted plants was recommended as way of damage assessment (Tarté et Pinochet, 1981). However, this approach may be useful only in areas where uprooting is the major consequence of nematode attack, which is not the case in West Africa for instance. In addition, uprooting may be due to other pests such as weevils or to edaphic problems. In Latin America, the ratio or the weight of functional roots are commonly used (Shillingford, 1988 ; Perez and Gomez, 1988). However, the definition of a functional root is not clearly established and its determination is highly subjective.

Gall index is widely used on crops susceptible to root-knot nematodes. But, symptoms on banana are usually light and atypical and may be confused with malformations, for instance because of soil compaction. Therefore, as well as for necroses due to migratory nematodes, a root section is needed for an accurate assessment of root-knot nematode attacks.

These direct methods of estimation are easy to use and enable an immediate field diagnosis. However, they have serious limitations linked with i) the subjectivity of estimation (e.g. Infestation level may be overestimated in a fairly undamaged field whereas underestimated in a heavily infested area), ii) their *post-mortem* characteristic (it is therefore too late to take action for the cycle in progress and, often, for that following).

These estimation methods are thus distinctly inadequate for experimental or monitoring purposes, and should only be used in special cases, when a more accurate assessment is not necessary or possible, for instance surveys in extensive cropping systems. For a more precise evaluation of nematode attack, identification and counting of nematodes are necessary.

ESTIMATION OF NEMATODE POPULATIONS

This estimation can be performed from plant tissues or/and soil samples. Analyses of soil samples are usually of little value for migratory endoparasitic nematodes, i.e. Pratylenchidae. A logarithmic correlation was found between soil and root populations of *R. similis* (Ambrose, 1984). However, the fitness of this relation is not accurate enough to estimate the root population from soil countings. For sedentary nematodes as well as for ectoparasites, counting of soil populations is necessary.

The reliability of counting is closely related to the efficiency of the following steps :

1) Field sampling ;
2) Extraction of nematodes ;
3) Counting.

Errors or inaccuracies, especially in sampling of aliquot parts during the successive steps of the whole process, may accumulate to generate an important bias.

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Sampling plant tissue.

The main problem is that nematode populations are usually distributed in highly variable spatial patterns whether in roots, plants or fields (Sarah, 1986 ; Hugon and Picard, 1988 ; Sarah and Perrier, 1988). As far as *R. similis* and banana are concerned, the standard deviation of individual sub-samplings is usually close to the mean, except for very high infestation levels. Therefore, sampling consideration is of very high importance for «catch efficiency» (Ferris, 1987).

There are two main questions :

1) Subsampling at the plant level,
2) Total sample size (number of plants - subsamples - to be sampled),

Regarding subsampling at the plant level, the main problem is the heterogeneity of the banana root system, with roots linked to different generations of plant parts (old plant, mother plant, ratoons). Dynamics of populations, infestation level and ratio between species at one sampling date in the roots are dependent on root links (Cadet and Quénéhervé, 1985 and 1986 ; Sarah, 1986). However, the global method developed by Vilardebo and Guérout (1974 a) is adapted to routine estimations such as in surveys and in field trials, because it is quite simple, non-destructive at plant level, and the resulting estimations are reliable.

However, more detailed investigations on plant-parasite relationships may need a more accurate method with separate - sectorial - samplings and countings, considering roots origin (Cadet and Quénéhervé, 1986 ; Sarah, 1986). This kind of sampling is highly destructive and much more intensive and, thus, cannot be used for routine purposes.

Considering sample size, and using a global sampling method, it was estimated that the optimal value is 15-20 banana plants for an «homogeneous» plot or plantation unit (Sarah and Perrier, 1988). A plot is considered to be homogeneous when bananas were planted at the same date and when no gradient or heterogeneity (soil, topography, plant development ...) can be seen. This optimal sample size is a balance between the need of accuracy and the amount of field work. In that case, the limits of the confidence interval of the estimated mean can be calculated using a factor of 1.3 (upper limit = m x 1.3 ; lower limit = m/1.3).

Routine estimations on experimental plots (36 to 60 plants) are done by sampling one in four plants (9 to 12 plants at each sampling date), every one or two months with a shift of one plant each sampling time. This means that sampling at time $t + 4$ will be performed on the same plants as sampled at time t. Nematode surveys on intensive cropping are performed on 15 to 20 plants per plantation unit (about one hectare). These plants are selected, whenever possible, at a definite phenological stage. Usually this sampling stage is flowering because i) it is easy to recognize, ii) there is generally a maximum level of infestation at this stage (Vilardebo, 1976 ; Hugon *et al.*, 1984 ; Sarah, 1986). In intensive cropping systems, this kind of sampling is not applicable due to the generally small number of plants. A qualitative survey, presence/absence of the nematode with eventually an indication of its abundance, may be sufficient but, generally, the ratio of sampled plants to total plants is high enough to give a quite good idea of the degree of infestation.

Extraction.

Direct counting of stained nematodes in plant tissue is possible. However, this technique is applicable only to very small samples which most likely will not be representative of the population because of the patchy spatial pattern (Merny and Luc, 1966). Therefore, nematodes must be counted from a larger (more representative) sample, from which they will be extracted.

Techniques of extraction can be divided in two categories :

1) Passive extractions, which do not require activity of the nematodes ;
2) Active extractions, requiring nematode mobility.

In passive techniques, nematodes are separated mechanically from their medium in two steps : dissociation (either dispersion of soil particles in water or maceration of plant tissue) then separation *sensu stricto* through density gradient (elutriation, flotation) and/or sieving. These methods are quite fast (especially when the flotation process is accelerated through centrifugation) and may give a fairly representative picture of the different species/stages (including eggs) actually present in the medium at sampling time whatever their mobility might be.

Errors or losses may accumulate during the successive steps. The dissociation may not be complete (especially if maceration is too short or too gentle) and/or some nematodes may be destroyed during the maceration process (especially if maceration is too long or too strong). The elutriation or flotation process may not separate nematodes completely from soil particles or tissue debris (especially if the dissociation was incomplete). Moreover, osmotic pressure of the solution may destroy some nematodes during the flotation process which must be shortened by centrifugation. Losses may also occur during the sieving process : part of the nematode population would stay in the upper sieve with the coarsest particles (especially if sieving time is too short) and another part would escape through the meshes of the lower sieve (especially if sieving time is too long). These losses can be limited by using meshes as small as possible. However, the smaller the mesh the weaker and the more exponentially expensive the sieve. Moreover, small meshes may be rapidly clogged up, especially by soil particles, during the process.

Among those techniques, centrifugal flotation (Coolen and d'Herde, 1972 ; Vilardebo and Guérout, 1974 b) is generally considered to be one of the best for extraction of deep endoparasitic nematodes from banana tissue. However, it is quite sophisticated and can be used only in well equipped laboratories. In more rustic conditions, the separation process may be simply performed by sieving alone. In that case, beside risks of loss, the counting of nematodes after sieving will be difficult because of the

generally large amount of particles present in the collected suspension (see below).

In active techniques, the mobile individuals/stages go out of crumbled soil or cut/macerated tissue by themselves. Nematodes passing through a filter (thin cotton or nylon cloth or muslin, retaining soil or tissue particles) are collected at the bottom of the apparatus. Oxygenation, which improves nematode mobility, thus extraction efficiency, can be done by bubbling, addition of H_2O_2, continual spray of a fine water mist, or simply by increasing the ratio surface/volume of water using a shallow dish. These techniques are generally little demanding in equipment and may be used even in a highly rustic environment. On the other hand, beside the fact that they are unable to extract non-mobile species or stages, a long period of time (up to several weeks) is needed to complete the extraction of the active population.

Importance of storage conditions.

If a large number of samples is to be analysed, the complete process may take a long time (several days or even weeks) which leads to a biased estimation, the population usually decreasing during storage. Low temperature, 10 to 20°C, can slow down the decrease. As shown by studies conducted in Guadeloupe, freezing of roots samples allows a long term storage with no detectable loss (Simon, unpublished). Since a 25-50 g aliquot part only is frozen, there is less of a problem of storage space requirement.

Counting.

Extracted nematodes are collected in 100 to 200 ml of water from which an aliquot part of only 1 to 5 ml will be used for counting. Thus homogenization of the nematode suspension is of prime importance. Since counting can be a drudger, human errors may occur. Reliability can be largely improved by good quality lenses, a suspension free of debris, monospecific population and a «reasonnable» number of nematodes (i.e. 20 to 100 nematode/ml). If the number of nematodes is to high, or too low, it will be necessary to dilute or concentrate the fraction, but this operation add one more error factor.

When extractions have been done only by sieving, the collected fraction will contain large amounts of debris. It is therefore useful to use methylen blue to differentiate nematodes from tissue debris.

CONCLUSIONS

Many sources of error occur and at each step of the process of estimation of nematode density. The reliability (accuracy and repeatability) of this estimation will depend on the care taken in realising the successive operations and on strict standardization of the techniques.

Sampled plants must be selected with non-subjective criteria : randomly or sequentially (e.g. one every four plants), on total plants or among those which are at a given phenological stage.

The number of sampled plants will depend on the size of the plot, the sampling technique but also on equipment and human resources. Generally below 15 plants, the confidence interval of the estimed population density increases sharply.

Storage of the samples must be for as short a time as possible and in environmental conditions that slow down plant decay and limit dehydration of samples. Freezing of the samples is recommanded whenever possible.

Extraction errors deal with i) selected aliquot parts of the field samples, ii) losses during the successives operations, iii) human errors. Sophistication and standardization of the extraction methods will reduce nematodes losses but may increase the risk of human errors.

Human errors are the major risk when counting. It is important that the operator is working in confort during this operation, so as to limit misevaluations.

Globaly, all these sources of error will accumulate and could lead to a highly biased estimation. Even if error and losses are minimized, an exact count of the population is impossible. However, respecting some basic rules (care and standardization) representative and repeatable estimations will be obtained.

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