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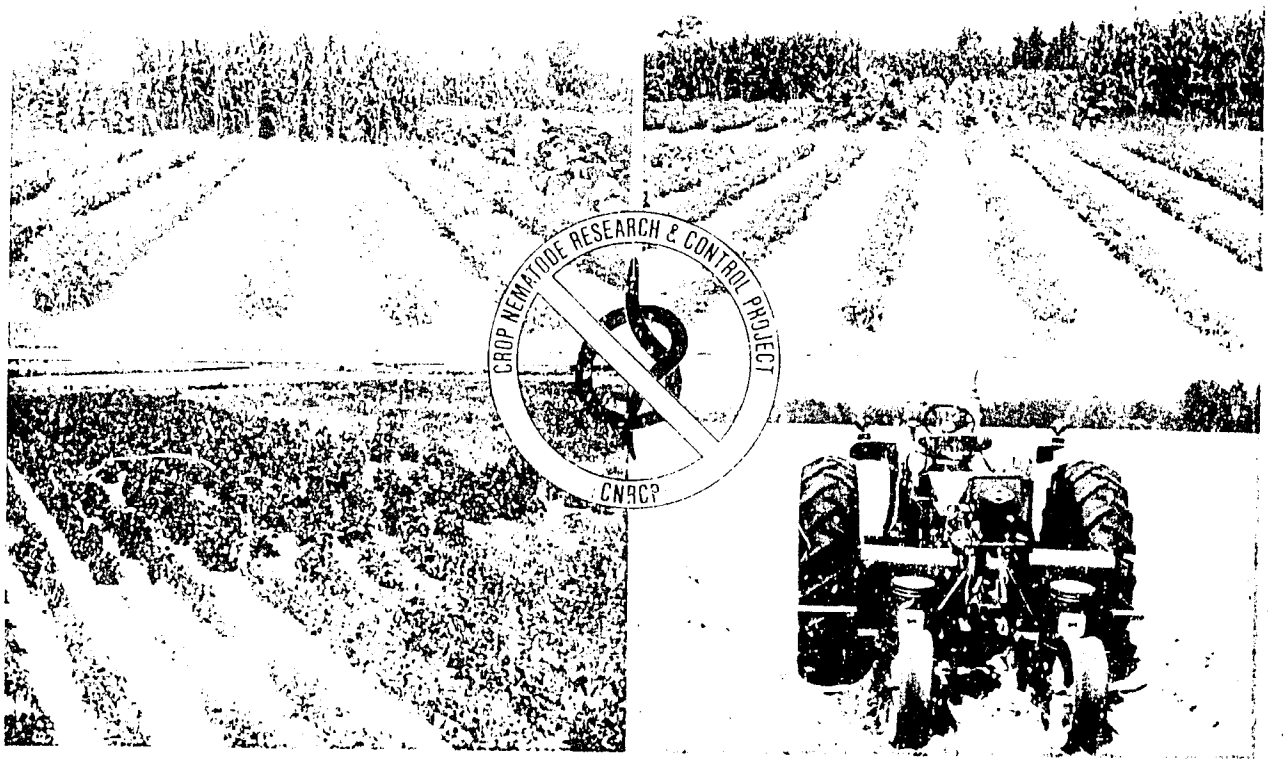
Cropping Systems Analysis for Limiting Losses Due to Plant-Parasitic Nematodes: Guide to Research Methodology



James P. Noe, Research Associate

Raleigh, North Carolina, USA
1986

Front cover: Four photographs demonstrating three management tactics for limiting losses due to plant-parasitic nematodes. Top two photos show small-plot crop-rotation research, with photos taken at same location, same number of days-after-planting, in consecutive growing seasons. Rotation of crops can be seen in the background, while effect of monoculture is evident in foreground plots (photos courtesy of C. J. Nusbaum). Bottom-left photo shows two different cultivars of soybean planted in a field infested with *Heterodera glycines*. Background block planted in a resistant cultivar is dark green with good plant vigor, demonstrating efficacy of resistance to nematodes compared with a susceptible cultivar in the foreground (photo courtesy of J. P. Ross). Bottom-right photo shows incorporation of granular nematicide into soil at planting time.

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Preface

The objective of this guide is to assist nematologists in lesser-developed countries in the design and implementation of cropping systems research programs for the limitation of crop losses due to plant-parasitic nematodes. The methods described represent our best effort at this time. However, systems research is an evolving area of investigation, and these methods will be updated periodically as improved approaches are developed. Cropping systems research is a primary goal of the Crop Nematode Research and Control Project (CNRCP), funded by the United States Agency for International Development through a cooperative agreement with North Carolina State University. The urgency of this research has been brought about by recent reductions in the number and availability of effective nematicides. Remaining nematicides are less effective and more costly, and their use may be prohibited in the near future because of environmental and health hazards. Also, the development and release of nematode-resistant cultivars has proceeded slowly. A comprehensive research program directed toward the improved deployment of crop rotations, available resistant cultivars, and other existing management practices is the surest and most practical approach to long-term increases in agricultural productivity. Optimization of existing management practices is the ultimate goal, including the improved deployment of crop rotations. This approach has the advantage of not requiring high-cost inputs from developing-country farmers.

This guide will be distributed to all cooperating nematologists of the CNRCP network. It is anticipated that Project staff and developing-country scientists will be involved at several levels of collaboration for the implementation of cropping systems research. As part of the planned cooperative research effort, the CNRCP cropping systems specialist will be involved in all phases of the design, implementation, and analysis of this research at a few selected international sites. These sites will be selected on the basis of local interest in cropping systems research, adequacy of local facilities, and the capability of personnel to carry out the research over a period of several years. At additional sites collaboration with the cropping systems specialist may be limited to close cooperation only during the initial design phase and final data analyses. Other cooperators may implement the methods outlined in this guide with Project assistance only in the data analysis phase, perhaps as a graduate-student thesis project. Regardless of the level of involvement by CNRCP staff, it is hoped that the distribution and use of this guide will stimulate interest, encourage discussion, and enhance the level of cropping systems research conducted by network nematologists.

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Cropping Systems Analysis for Limiting Losses Due to Plant-Parasitic Nematodes: Guide to Research Methodology

INTRODUCTION

Plant-parasitic nematodes are a severe constraint to agricultural production in developing tropical and semi-tropical countries. Although there has been an increasing appreciation of the damage caused by these pests in recent years, during the same time period there has been a substantial loss in the availability of effective nematicides due to environmental hazards associated with their use. Those nematicides that remain on the market are less effective and continue to increase in cost, while farm revenues decrease.

The use of cultivars resistant to various plant-parasitic nematodes offers great promise for the future, but at the present time this management alternative is only a promise for most nematode-host combinations. Where resistant cultivars have been deployed, their effectiveness has been rapidly reduced by the emergence of new host races of the target nematode species, or by the emergence of new infestations of different nematode species on the resistant hosts.

Biological control agents also offer promise for the control of losses due to plant-parasitic nematodes, but their development and deployment is proceeding slowly because the detailed ecology, production, and formulation of each agent must be studied. Also, it appears that biological control agents will not provide suitable levels of control alone but must be incorporated into other management schemes. Therefore, at this time, the optimal deployment of remaining nematicides and resistant cultivars is critical to successful, sustained management of crops to limit losses due to plant-parasitic nematodes.

Crop rotation is a management technique which is particularly suitable for use in developing areas of the world. Sequential alternation of crops with varying degrees of susceptibility to the nematode pest can produce striking results in the reduction of crop losses (Fig. 1). The deployment of optimized crop rotation regimes requires no high-cost or high-technology inputs, and the concept of rotating crops is a part of the agricultural history of many subsistence-level farming operations. In this guide, an emphasis is placed on crop rotation as a management tool since in many areas with severe nematode problems nematode-resistant cultivars are not available, and local economic conditions do not allow the use of costly treatments. The analysis of cropping systems as they relate to plant-parasitic nematodes will provide information necessary for the optimal deployment of all existing management techniques. The specific objectives of this research are:

1. To provide farmers with optimum crop rotation/management sequences for limiting crop losses due to plant-parasitic nematodes.
2. To quantify the relationships of nematode numbers to crop yields for a variety of crops and plant-parasitic nematodes of worldwide importance.
3. To demonstrate the feasibility of optimizing cropping systems for farmers through an international cooperative research effort.

A study of cropping systems includes quantitative analyses of the relationships among crops, pests, and management techniques which are deployed or deployable in the target system. In order to provide comprehensive information for management-decision systems, research designs must include economic and social aspects of the farming situation. Ideally, even livestock operations should be included in the economic analysis of cropping systems. As an example of how livestock and crops can interact, it is conceivable that the most profitable management alternative for a particular plant-parasitic nematode infestation would be to use the land as pasture for a number of years, thus rotating to a profitable "non-host." Although comprehensive information is needed, specific research designs for the analysis of cropping systems may be limited to selected components. Additional components can then be added to the system as key relationships are quantified.

This guide describes a methodology for analysis of cropping systems aimed toward limiting losses due to plant-parasitic nematodes. The proposed research design integrates a relatively simple field-oriented approach to data collection with a more sophisticated structure of mathematical modelling, computer simulation, and linear optimization. Field-plot design and data collection are described in detail, and a step-by-step overview of the mathematical processes is presented. Mathematical discussions are necessarily limited and meant to provide the researcher with a general idea of the cropping systems analytical approach. This research should not be attempted without the assistance of a professional biomathematician. Services related to the analysis of nematode cropping-systems data are being offered to cooperators in the Crop Nematode

Fig. 1. Effect of rotating to a nonhost (left) and host (right) in the previous year, showing much less damage caused by *Meloidogyne hapla* on the current crop of peanuts. (Photo courtesy J. N. Sasser).

Research and Control Project (CNRCP) by the cropping-systems specialist.

A key component of this research is quantification of the relationships of nematodes to their hosts. These relationships are affected by other pests in the cropping system, and eventually these effects must be considered as part of the nematode-management system. Although the methods described in this guide are for studying nematode-host relationships only, other pest models can be incorporated into the system at a later date. After accurate derivation of the appropriate nematode damage functions and reproductive curves through this research, the mathematical functions representing these relationships could be modified to reflect the impact of other pests as information becomes available. It is anticipated that the research methods presented in this guide will be periodically upgraded as our understanding of the complexities of cropping systems increases.

LITERATURE REVIEW

Comprehensive studies of cropping systems have evolved from a number of related areas, including the analysis of crop rotations, the development of quantitative host-parasite relationships, nematicide and resistant-cultivar evaluations, and integrated pest-management research. It would be difficult to

review the literature extensively in all related areas, but some background information is necessary in crop-rotation analyses and in the quantification of nematode-host relationships.

In 1911, Bessey (7) suggested the use of certain crop rotations to minimize losses to root-knot nematodes in susceptible vegetable crops. Since that time numerous studies have been done on crop rotations to manage populations of plant-parasitic nematodes (1, 9-15, 19, 20, 22-26, 28-30, 32, 36-39, 42, 44, 45). The 26 papers listed represent only a sampling of more than 200 citations produced by a literature search on nematode crop-rotation research. These reports have established that the selection of appropriate cropping sequences can be an effective tool in the management of nematode populations, and specific crop rotation results are available in the papers cited. This guide, however, is for the development of methodology, and therefore the methods employed by previous researchers are of primary interest.

End-of-season, or end-of-rotation, nematode counts have been the variables of interest in most crop-rotation experiments (1, 10-14, 19, 20, 25, 26, 28, 30, 36-39, 42, 45), with yield data (15, 22, 23, 29, 44) and nematicide results (9, 23, 24, 32) also included in some papers. End-of-season counts were deemed important as an indication of whether or not the population had been reduced to a level that would not cause economic damage in future crops. In designing the field research, the researcher usually selected a promising series of rotations based on previous information, and each rotation selected was implemented in field plots of various sizes. Final population counts and yield then were compared across rotations to determine the most appropriate management sequences. This data-collection method was an efficient way of studying rotations in the field; however, the rotations analyzed were limited to those selected by the researcher. The effectiveness of the resulting recommendations were contingent on identification of the best cropping sequences by the researcher prior to beginning the research.

These studies also were limited in the number of growing seasons which could be analyzed in the field due to time limitations on the research programs. The time and number-of-crop constraints were not a problem in cases where only two or three crops were included in the research design for only two or three years, but effective management of nematode problems for optimum net production and/or profit may require the analysis of five or more crops for five to ten years. Additional crops could not be added to these analyses at a later date since each crop rotation had to be included in the original research design.

Concurrent with the development of crop-rotation research has been the development of methods for quantifying the relationships of plant-parasitic nematodes to their hosts. Particular emphasis has been placed on the development of nematode damage functions, primarily through the fitting of various mathematical functions to experimental data. In order to assess the value of having reduced the nematode population to a certain level, one must project the damage caused by that population of nematodes to the target crop. Relating planting-time nematode numbers to crop damage has been the foundation of nematode advisory services (3).

Plant-parasitic nematodes are relatively immobile in their soil environment, so that crop losses are largely dependent on pest pressures present at the beginning of the growing season, modified to varying degrees by certain environmental variables. In 1965, Seinhorst (43) proposed a descriptive model for the relationship of nematode density to plant growth which has been further refined and expanded (16, 17). The use of Seinhorst damage functions has been well documented (4, 16). A logistic model (27) has been developed to represent the relationship between nematode density and root damage, and linear functions fitted to log-transformed data (4, 5, 41) also have been used to represent nematode damage functions. All of these models incorporate the basic biological concept that damage per parasite decreases as density increases.

Another important consideration in the analysis of cropping systems is the reproduction of plant-parasitic nematodes under various crops and management practices. The rate of reproduction will influence levels of residual nematodes which, in turn, will impact future management decisions. The relationship of end-of-season nematode counts to preplant counts has been described by a logistic mathematical function (27). Where log-transformed population data were analyzed, linear reproductive curves were derived. Both logistic and log-linear curves represent decreasing fecundity per female as initial density increases.

The next step in the development of cropping systems analysis for nematode management has been the merging of crop rotation studies with the derivation of quantitative host-parasite relationships, for the purpose of simulating the effects of cropping sequences on nematode populations. A model has been developed (27) from previously published information to simulate the population dynamics of *Globodera rostochiensis* on various host and nonhost crops by combining reproductive curves, damage functions, and between-host survival functions. In another study (15), using small-plot experimental techniques, the effects of *Meloidogyne incognita* on a cotton-cowpea cropping system was simulated. In that work, crop damage, nematode reproduction, and between-crop survival functions were combined

with economic analysis of crop values and management costs to simulate the net profitablity of selected management practices.

Simulation of crop-nematode interaction can be a useful tool in understanding the impact of various crop sequences and management practices on nematode population dynamics and on the economic analysis of a production system. Further research needs to be directed toward the implementation of this potential in an applied diagnosis and management recommendation service, with decisions based on expert-information systems using quantitative host-parasite relationships and comprehensive economic models.

METHODS FOR NEMATODE CROPPING SYSTEMS ANALYSIS

A methodology is described for the systematic quantification of nematode-host relationships in small experimental field plots. A structure will be developed for integrating this information with economic and cultural variables into a representation of the target cropping system, which then can be used to recommend optimum cropping sequences and management practices.

Site Selection

Within each agricultural area to be studied, a research site must be selected where there is a high infestation of the target nematodes, and where a sufficient range of crops is commonly grown to allow for the analysis of complex long-term rotations. Portions of the research site must be severely infested with nematodes since an objective of the research is to quantify the nematode damage function. In order to quantify the entire function, the research plots must include areas with minimum yield or highest damage levels. Much time and effort can be wasted by attempts to derive nematode damage functions in areas where the nematode pressure is not high enough to cause significant damage. Likewise, there must be a number of crops of varying susceptibility to the nematode pests to select from when the cropping sequence is optimized, or else the eventual recommendations will be trivial.

Approximately 0.25 hectares should be allotted for each crop to be established at a site. Replications of the research sites may be located in different fields, or several sites can be located in one large field. In order to analyze three crops, with three replications of the sampling area per crop, a total of 2.25 hectares would be needed. A cropping systems research site can be located in part of a larger field, with no disruption of normal cultural practices.

Sites may be located in farmers' fields where the researcher has a close working relationship with the farmer and is confident that good crop management practices will be implemented. Sites also may be located on institutional research stations, where the implementation of good crop management practices can be controlled by the researcher. Since the primary goal of this phase of the research is to derive nematode-host relationships, the crop must be properly managed to ensure that nematode effects will be accurately represented in the data. The capability of the researcher, farmer, or production manager to manage the crop properly is of overriding importance in selection of a cropping systems research site. Since specific rotations will be implemented in the field plots, the researcher should be sure of access to the same sites for a number of years.

Crop/Management Selection

Crops should be selected for analysis that are appropriate for the research sites and for the local farming systems. A selection of four to five annual row crops, including nonhosts and cultivars resistant to the target nematodes would be ideal. The techniques described in this guide for quantifying and optimizing cropping systems are best applied to annual row crops planted in sequential monocultures. Techniques have not yet been developed for quantifying the effects of plant-parasitic nematodes in intercropping systems, and the concepts of optimized rotations are difficult to implement in long-cycle perennial crops. In the choice of specific crops, the feasibility of harvesting small plots should be considered. In four-row plots, only the two center rows may be harvested, as is common in other areas of agricultural research. Where a broadcast or continuously planted crop is selected, harvesting small plots may be more difficult. Crops that must be harvested mechanically may require larger experimental plots than are ideal for the collection of nematode population data.

Alternative management practices, such as the application of nematicides and biological control agents, can be implemented as part of the cropping systems research design. The addition of these factors will increase considerably the size and number of sites needed since a factorial design will have to be established with appropriate replications. Each crop for which the use of nematicides or biological control agents is being analyzed will have to be established both in fields that are treated and in those that are not treated. It should be well documented by the researcher that the alternative control methods are commonly practiced in the cropping systems under study, or that the introduction of methods would be feasible if their use were indicated by the cropping systems analysis. All possible management alternatives should eventually be investigated, however, since economic analyses and the selection of opti-

mum sequences will be more meaningful if there are more possibilities from which to select.

Establishing Plots

A systematic sampling grid will be constructed immediately after planting at each of the research sites. The sampling grid will consist of 64 to 100, 24-m^2 plots, laid off along plant rows, with no alleys between plots (Fig. 2). This design is meant to reflect as closely as possible actual grower practices and to allow placement of the grids in farmers' fields without disrupting normal operations. Each plot should be approximately 6 m long, and four plant-rows wide (approximately 4 m). This grid can be established by placing 25- to 45-cm high wooden stakes marked with plot numbers in the center of plant rows (Fig. 3). Placing relatively short stakes in row centers will allow for normal cultivation procedures without the stakes being disturbed. All that is needed for establishment of the grid is a measuring tape and a supply of wooden stakes. The grid should be as nearly square as possible. A grid with 64 plots should be arranged eight plots wide (total of 32 plant rows), with eight plots in each row. Permanent markers should be established outside the area of cultivation, so that plots can be located in subsequent growing seasons.

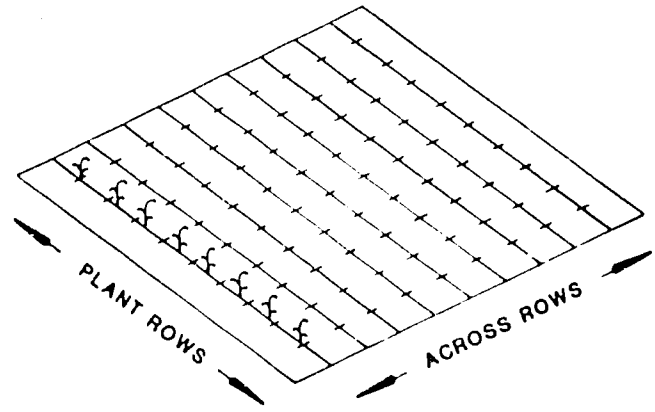

Fig. 2. Diagram of systematic sampling grid laid out along plant rows for collecting cropping systems research data.

Fig. 3. Cropping systems sampling grid marked off with 30-cm wooden stakes in plant row centers. Placement and height of stakes will allow normal cultivation and management of the crop.

It is important that the sampling grid be extended across a number of plant rows since this will maximize the variation among nematode counts (35). Precise placement of the grid at the research site is critical and should be based on a preliminary nematode assay. Plant-parasitic nematodes usually occur in clusters within infested fields (Fig. 4) (21, 31, 33, 34, 35), and the cropping systems research is designed to make use of this spatial pattern. One side of the sampling grid should be located directly over a high population density cluster of nematodes. Usually, over a distance of 20 to 30 m across plant rows, the nematode densities will vary greatly, and the other side of the grid will have areas with very low to zero nematode counts (Fig. 5). With this design, a sufficient range of nematode densities will be included in the plots to allow for the derivation of nematode damage functions and nematode reproductive curves (Fig. 6). This wide range of initial densities is essential to the success of the cropping systems experimental design. Cropping systems research sites should not be located where the infestation of nematodes is too uniform or where other constraints will not allow the grid to be constructed as described. In such a situation, another site should be chosen. Another nematode-host system may have to be analyzed if the nematode characteristically has a uniform spatial pattern in the field. If the nematode spatial pattern were too uniform, then there would not be sufficient variation in the population densities among the individual plots for the determination of host-parasite relationships.

Sampling For Nematodes

Collecting soil samples and assaying the soil for plant-parasitic nematodes is perhaps the most arduous and error-prone part of this research. Samples must be collected in such a way that they accurately represent the nematode population, and assaying methods must be standarized to the extent that their results are predictable and can be interpreted by other researchers. The entire process must be prac-

Fig. 4. Patchy distribution of damage caused by *Belonolaimus longicaudatus* on soybeans.

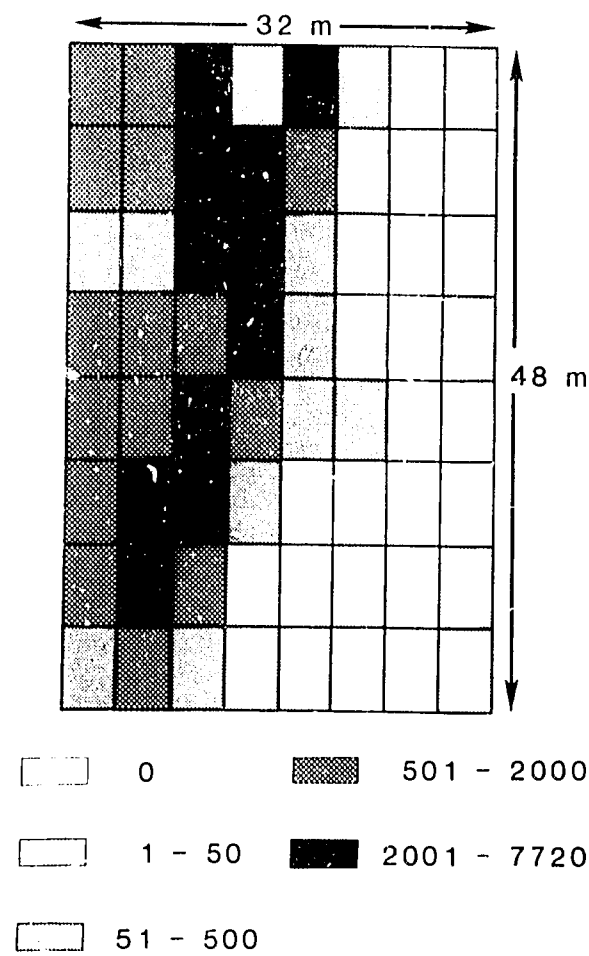

Fig. 5. Distribution of *Meloidogyne hapla* counts from cropping systems research plots. The sampling grid was placed so that a major cluster of nematodes was located on one side of the grid, whereas there were very few or zero nematodes on the other side.

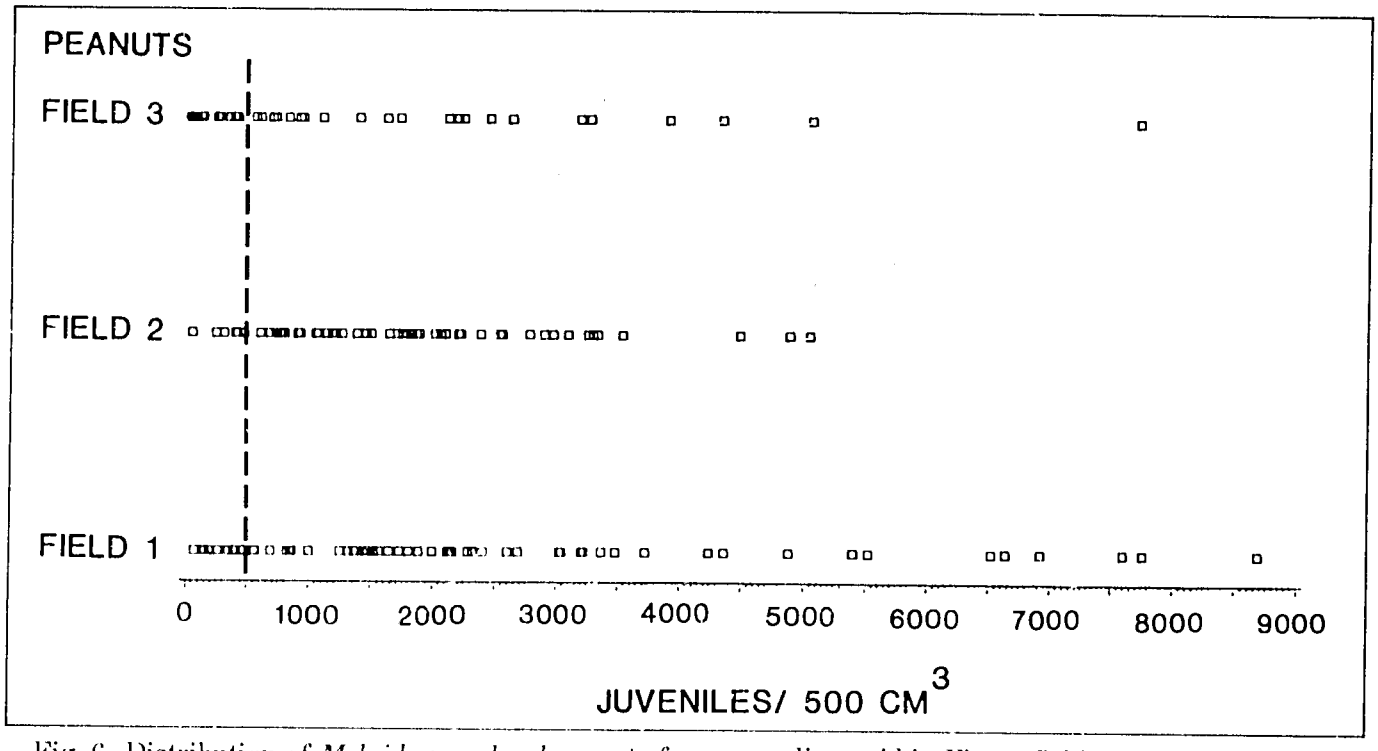

Fig. 6. Distribution of *Meloidogyne hapla* counts from sampling grid in Fig. 5 (field 3), along with counts from two other research sites, showing wide range of counts well distributed around the threshhold level (indicated by vertical dashed line).

tical and consistent with methods currently available or implementable in nematode diagnostic labs. Since the eventual diagnosis and recommendation service will be based on assay results, the nematode counts used in this research must coincide with counts that may be expected from a nematode assay service.

The factors of accuracy and consistency must be balanced against the time and effort expendable by the researcher in cropping systems research, along with the availability of skilled technical personnel and laboratory equipment. Where the cropping systems research design includes simultaneous analysis of three crops, with three replications of the sampling grid per crop, a total of 576 soil samples (64 small plots per grid) would have to be collected and processed at each sampling date. Assessment of initial and final nematode populations (two sampling dates) would require 1,152 samples per growing season. The number of samples per year can be reduced, however, by limiting the experimental design to analysis of the nematode-host relationships of only one (384 samples) or two (768 samples) crops during each growing season and extending the research over a greater number of years.

There is no single best method for taking soil samples and extracting plant-parasitic nematodes, and any procedure will need to be adapted for local expertise and crop management practices. However, certain simple, standarized methods are applicable to many species of plant-parasitic nematodes.

Plots should be sampled at planting time and at harvest time so initial (P_i) and final (P_f) population densities can be estimated. Ten to twenty 2.5-cm-diameter by 20-cm-deep soil samples are removed from the center rows of each plot in a zigzag pattern, with the cores spaced equally along the plant rows (Fig. 7). The individual cores from each plot are bulked for extraction of nematodes and counting. Soil samples to be used for nematode assay are perishable and should be handled properly. Samples should be collected in plastic bags to prevent drying. Bags should not be left in direct sunlight, as resulting high temperatures may decrease the viability of nematodes in the sample. Ideally, samples should be processed within one week of the date of sampling, but where this is not a feasible time period, storage of the samples at 10-15°C will prolong the recoverability of many nematodes species ([2]).

For extraction of motile life stages, such as the infective second-stage juveniles of root-knot and cyst nematodes, Baermann methods can be used (2) (Fig. 8). The Baermann funnel technique requires a supply of funnels (10-20 cm diameter) with rubber tubing attached to the stem and a clip to close off the tubing. The funnel is placed on a stand and filled nearly to the top with water. A 20-mesh plastic or stainless steel screen (do not use copper mesh) is placed in the funnel at water level. A 20- to 30-cm^3 aliquant of soil is spread on a wet-strength paper tissue and placed

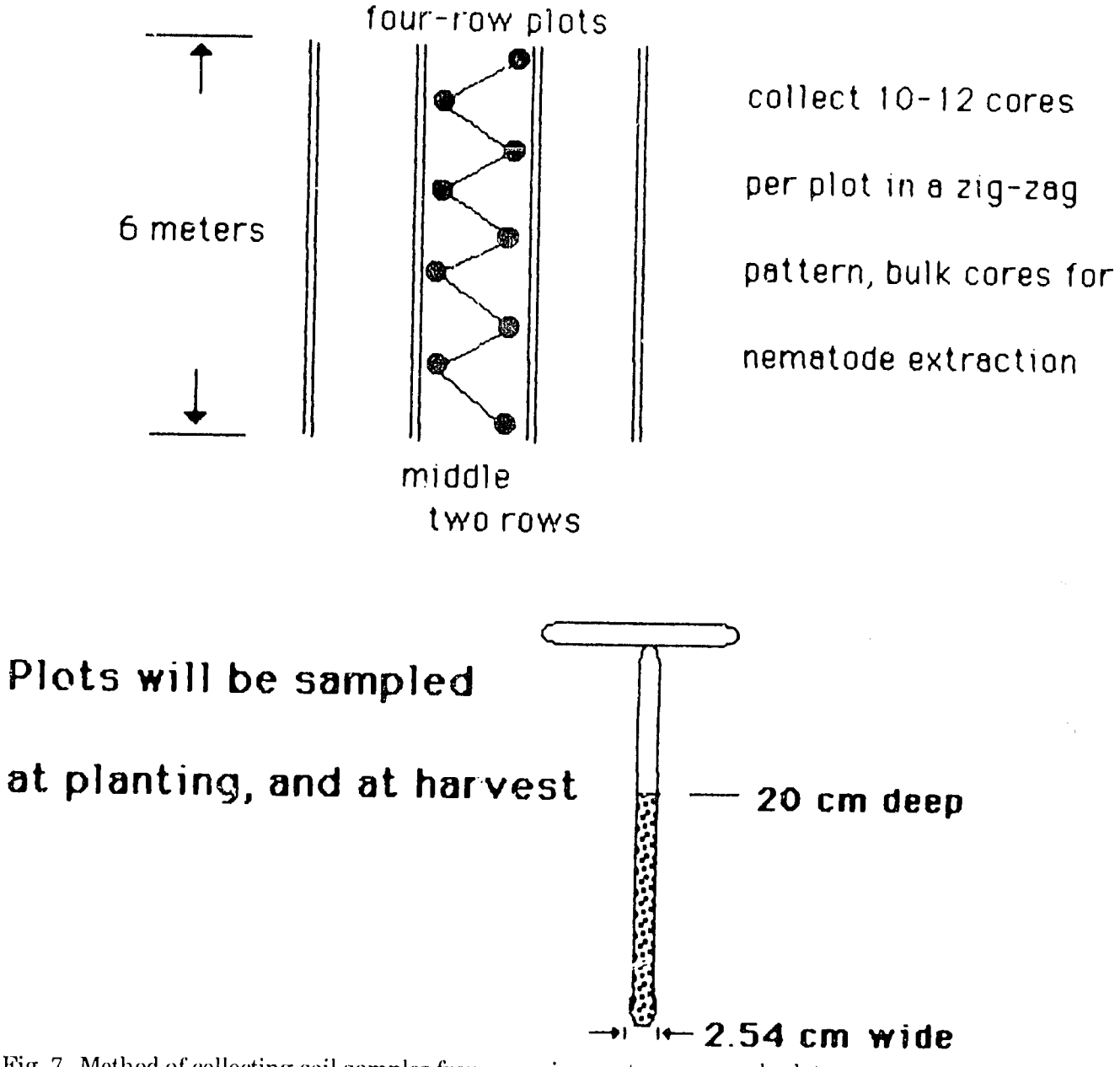

Fig. 7. Method of collecting soil samples from cropping systems research plots.

on the screen. Add water just to cover the soil, and incubate the samples for 48 h at room temperature (21-24°C is ideal). The nematodes then are removed from the bottom of the funnel by release of the clip on the plastic tubing. Plant-parasitic species are then identified and counted (Fig. 9).

The soil sample must be mixed thoroughly, but gently, before the aliquant of soil is removed for Baermann extraction. Rough handling of the sample will decrease the recoverability of certain nematode species. The number of nematodes recovered by this technique is sensitive to temperature and the length of time incubated. Time of incubation should be monitored closely, and ambient temperatures should be recorded for later reference. During the incubation period, motile nematodes will move out of the soil through the tissue and screen and collect at the bottom of the funnel. When Baermann methods are used, care should be taken to ensure that proper amounts of soil and water are added. Too much soil or water will greatly reduce the efficiency of this extraction technique.

For the extraction of non-motile life stages, other techniques will have to be considered. Flotation-sieving, centrifugal-flotation, and elutriation may all be used for non-motile nematodes (2). In some cases, where critical population densities may be extremely low, or where the nematodes are primarily in the egg stage, bioassay techniques may be required (2). Regardless of the extraction technique employed, the researcher should describe the methods in detail and determine the efficiency of the tech-

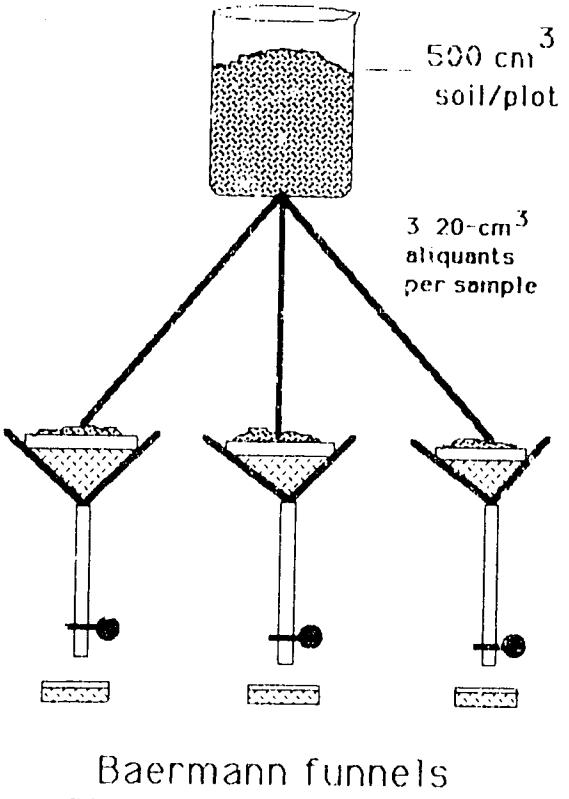

Fig. 8. Diagram of Baermann funnel technique for extracting motile plant-parasitic nematodes from soil samples.

nique under local conditions. Efficiency may be determined by adding a known number of nematodes to a given volume of soil, and processing the sample through the extraction technique. The actual number of nematodes recovered is compared to the number added to estimate efficiency. This information will enable other researchers to interpret and utilize the data.

Harvest

The process of harvesting will probably cause the greatest disruption of normal grower practices, since yields will have to be assessed in each of the small plots. All crops should be harvested when fully mature and at an appropriate stage of field dryness. Close coordination with the farmer or production manager will be required so that ideal harvest dates can be determined. Only yields from the two center rows of each plot need be assessed, although whole-plot yields are acceptable if harvesting all four rows is more convenient. Usually, small plots can be harvested manually, but mechanical harvesting may be desirable for some crops. Care should be taken to ensure that the yields from each of the plots are kept separate if a mechanical harvester is used.

Where quality of the crop is of economic concern, the harvest from each plot will have to be graded. Plant-parasitic nematodes may fail to cause a quantitative loss but may significantly damage the marketability of a crop. This market-quality effect is usually found with root crops, such as carrots, peanuts, and potatoes. Any grading systems employed in the cropping systems research will have to be converted into a quantitative measure for data analysis.

DATA ANALYSIS

After initial population (P_i), final population (P_f), and yield (Y) data are collected from each of the experimental plots, information expressing nematode-host relationships must be extracted, reduced, and represented by characteristic mathematical functions. The basic approach will be to fit appropriate curves to the data through best-fit empirical techniques. The best function for simulating the cropping system will be that function which best decribes the particular set of data, not necessarily the most attractive biological or theoretical model. After derivation, the functions will be combined with economic and cultural variables into a constraint analysis model, which will be optimized through linear programming techniques.

Nematode Damage Functions

Planting-time nematode assays and yields measured in each plot are used to determine the relationships of crop yields to initial nematode densities. Nematode damage functions are usually represented

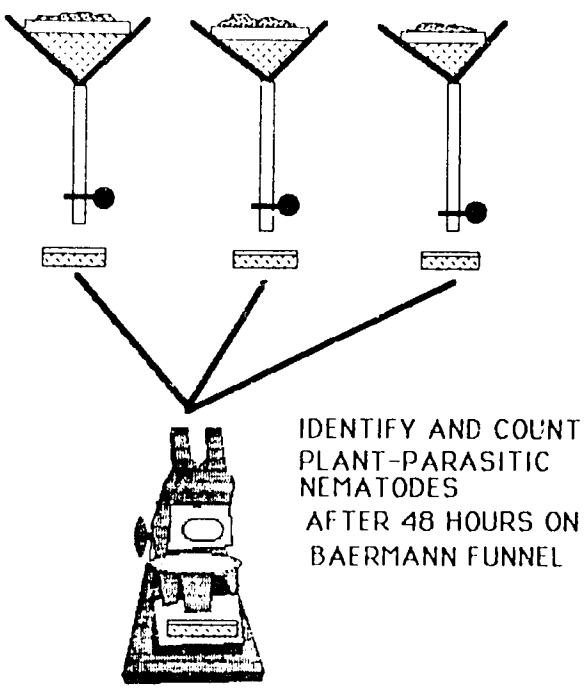

Fig. 9. As part of the research design, nematodes should be removed from the Baermann funnels after a specified length of time (48 h), identified to species and counted.

by a negative logistic relationship, with a relatively level initial phase, then rapidly declining yield as nematode density increases, followed by another levelling off, or asymptotic approach to a minimum yield (Fig. 10). Several alternative models are available to describe this relationship between initial nematode counts and yield. In the simplest case, nematode counts are transformed to a logarithmic scale, at which point the relationship may be more or less linear over most of the counts. A linear model can then be fitted to the curve with a computer program for general linear analyses (8, 40). The fit may be poor at extreme ends of the curve where damage is not measurable, or after minimum yield has been obtained. This minimum may be zero yield, but there still must be some point at which decrease in yield levels off, or becomes asymptotic. Most of the variations in nematode damage functions have been attempts to fit the curves to the extremes.

Seinhorst (43) used a discontinuous model, with a straight line up to the point where yield loss becomes measurable, and then a negative exponential model to describe the rapid decrease in yield and asymptotic approach to a minimum. This curve is difficult to deal with mathematically since it is discontinuous and can only be fitted to data through iterative methods (18) or by visual estimations of parameters. An alternative approach to fitting the entire curve would be to describe a basic "S"-shaped negative-logistic function and estimate the parameters with a computer program for nonlinear curve fitting (8, 40).

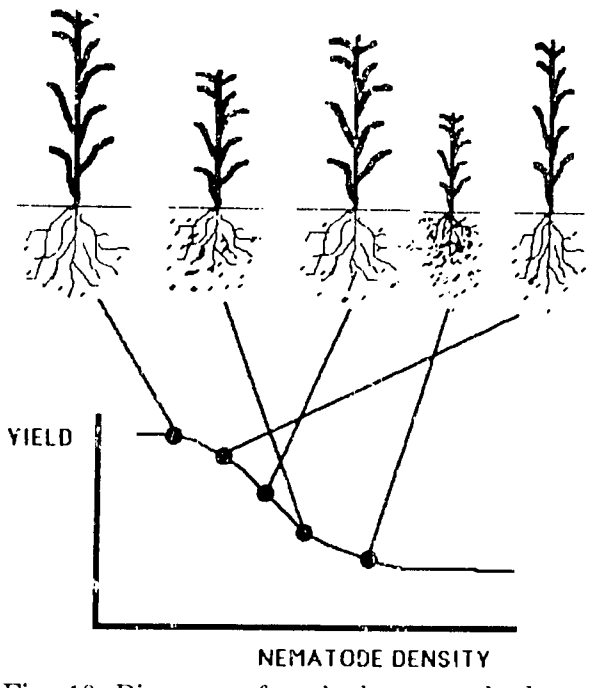

Fig. 10. Diagram of typical nematode damage function, showing variable response to increasing densities.

Any of these models can adequately describe the relationship between initial population and yield. Given the amount of experimental and sampling error in nematode field plots, there is probably very little justification to recommend one over the other from a statistical viewpoint. The most important consideration is that the function perform accurately in critical economic decision areas of the curve. If a critical economic decision threshhold lies near an extreme end of the curve, then the simple log-linear model may not be adequate. If that model is accurate, however, its use would be indicated by the relative simplicity of its statistical estimation and interpretation. The data from each nematode-host combination will have to be examined so the most appropriate model can be determined.

Different management practices will be represented in the systems analysis as modifications of the damage curves for particular nematode-host combinations. Use of a chemical nematicide should decrease the slope of the curve, as the basic relationship of planting time densities to crop yield is altered. Also, addition of a biological control agent would change the curve, perhaps altering the shape as well as the slope. Entire grids treated with each management tactic to be analyzed must be placed in fields in a replicated design so that damage functions characteristic to each method can be derived and compared to curves from untreated grids.

The importance of appropriate placement of the cropping systems research grids should now be apparent. A wide range of initial nematode densities must be present in the plots in order to provide information on the entire nematode damage function. If only low pathogenic densities are represented, then only the left side of the curve may be derived, and there will be no indication of maximum damage. Likewise, if only high pathogenic densities are recorded there may be no indication of critical threshhold levels. Population levels which represent high or low pathogenic densities will differ greatly for each nematode-host combination, and it will be up to the researcher to select levels centering on critical threshholds for the primary nematode-host association in the system (Fig. 6). This selection process, which is critical to the success of the cropping system analysis, will have to be based on experience, farmer input, and previous research.

Biological and environmental factors also affect the relationship of crop yield to nematode densities. Other nematodes may contribute to total yield loss and may interact with damage resulting from the target species, either by inhibiting or enhancing the expression of damage. Likewise, edaphic factors may greatly alter damage relationships. Any of these factors can be incorporated into nematode

damage functions through the use of mutivariate analyses. Instead of simple linear regression, multivariate regression can be used to quantify the impact of many nematode species simultaneously, along with soil texture, moisture, and any other quantitative variable measured in each of the field plots. Before incorporating these factors into the damage model for this research, however, the researcher must determine the likelihood of this related information becoming a part of the nematode diagnostic-advisory recommendations. An advisory recommendation based on soil texture, soil moisture, and other edaphic parameters will not be useful unless edaphic data is available with each nematode sample. However, information on other species of plant-parasitic nematodes is usually available with an advisory sample, so it is essential that interspecific relationships be analyzed in the cropping systems research.

The effects of large-scale climatic variation on nematode damage functions can be accounted for in the cropping systems research design. The research will be implemented over a number of growing seasons, including unusually wet and unusually dry, or cold and hot seasons. The variation in parameter estimates for the yield-loss models will provide an estimate of the confidence intervals that may be placed around damage functions. These confidence intervals will indicate how much variation to expect in the accuracy of the system based on year-to-year changes in the weather. For irrigated production systems this effect should be minimal, but for rainfed systems it may be quite large.

More detailed studies may be done in the greenhouse or in microplots to estimate the effects of certain edaphic parameters on damage functions. Once the basic function has been derived in field plots, it may be modified according to relationships derived from factorial experiments with varying levels of moisture, soil textures, or nutrients interacting with various nematode densities. Interactions of different plant-parasitic nematodes may be studied in the same manner. Each of these effects should be represented by either a change in the horizontal location of the curve, a change in the slope of the curve, or a change in both parameters. By examination of extreme levels that are likely to occur in the field, the amount of variation in the models attributable to each characteristic can be determined and factored into the predicted confidence intervals. Specific levels of each characteristic studied can be adjusted to represent geographical differences, such as predominantly sandy soils versus clay soils, or wet verus dry areas. The derivation of nematode damage functions for use in the cropping systems analysis will be viewed as an on-going process, with the accuracy and precision of the functions continually improved as more information is made available.

Nematode Reproductive Curves

Nematode population growth over a growing season can be expressed by relating end-of-season nematode counts to initial counts with an appropriate mathematical function. Usually, a suitable function will be one which incorporates density-dependent rates of reproduction. The rate of population increase is very high at low nematode densities but levels off as the ecosystem becomes overpopulated until there is no further increase (Fig. 11). The individual cropping systems research plots are sampled at the beginning and end of the season so that the reproduction of the target nematodes on each crop in the sequence can be estimated. As was the case for nematode damage functions, there are several methods by which the relationship of end-of-season nematode densities to initial densities can be quantified. The simplest relation is defined by a logarithmic transformation of both P_f and P_i, followed by estimation of the parameters of a linear model. A logistic model may also be fitted to transformed or actual data (27).

The estimation of parameters in nonlinear formulations is more involved and more difficult to interpret, but the logistic function will probably describe the extreme ends of the curve more accurately. Depending on the sequence of crops in the cropping system and overall population density, accuracy at those portions of the curve may not be critical. The data should be examined for each nematode-host combination, and the simplest function which provides sufficient accuracy should be used to describe the nematode reproductive curve. Estimation of parameters for the linear and nonlinear models will be done with statistical computer programs (8, 40).

Cropping System Analysis

After characteristic curves for crop damage and nematode reproduction have been derived, the resulting series of mathematical functions, along with appropriate control structures, will be programmed into a simulation of crop sequences. The analysis can best be viewed as an iterative process, proceeding in discrete steps from growing season to growing season, with the crop to be grown during each season represented by its characteristic curves (Fig. 12). A sequence of crops/management practices and initial nematode densities are input to the iterative portion of the program. The initial density is then used in crop loss and reproductive functions for the first crop in the sequence to project a final density and a crop yield, which is then translated to a crop value based on local currency. The projected final

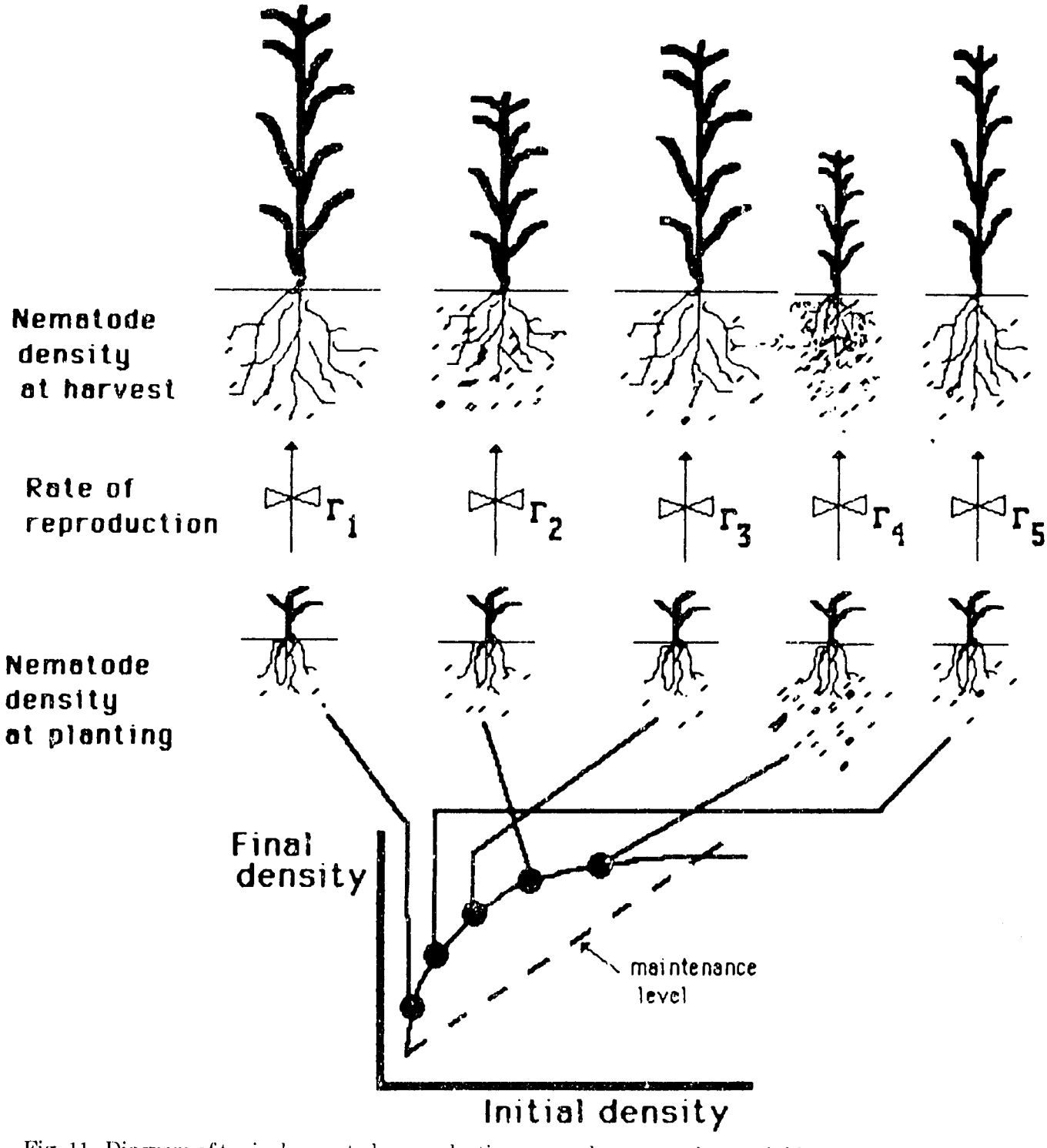

Fig. 11. Diagram of typical nematode reproduction curve, demonstrating variable rates at differing densities, and decreasing fecundity per female as density increases and the crop damage is greater.

nematode density for the first growing season, modified by appropriate between-crop survival functions, is then used as an initial density for the next crop in the sequence. The control structure of the computer program (Fig. 13) steps through the entire cropping sequence in this fashion, finally outputting the profitability of the selected sequence.

The iterative computer simulation can analyze a large number of crop/management sequences in a relatively short time. However, the system can be improved by the use of quantitative decision-management tools to select only those sequences that are optimal for a given set of constraints. The need for optimization techniques is evident when one considers a system with five different crop and management-alternative combinations to be simulated over

a period of 10 years. If all possible combinations and permutations are included, there are 9,765,625 possible cropping sequences to consider. In addition to problems associated with the overwhelming num- involved in this system is the additional complication of proper quantification and application of crop production constraints, as well as social and cultural farming-system constraints. The resulting multiple-constraint crop production system can be analyzed comprehensively with a linear programming model for decision making.

Linear programming is a mathematical technique for improving decision-making information available to managers (6). The technique consists of

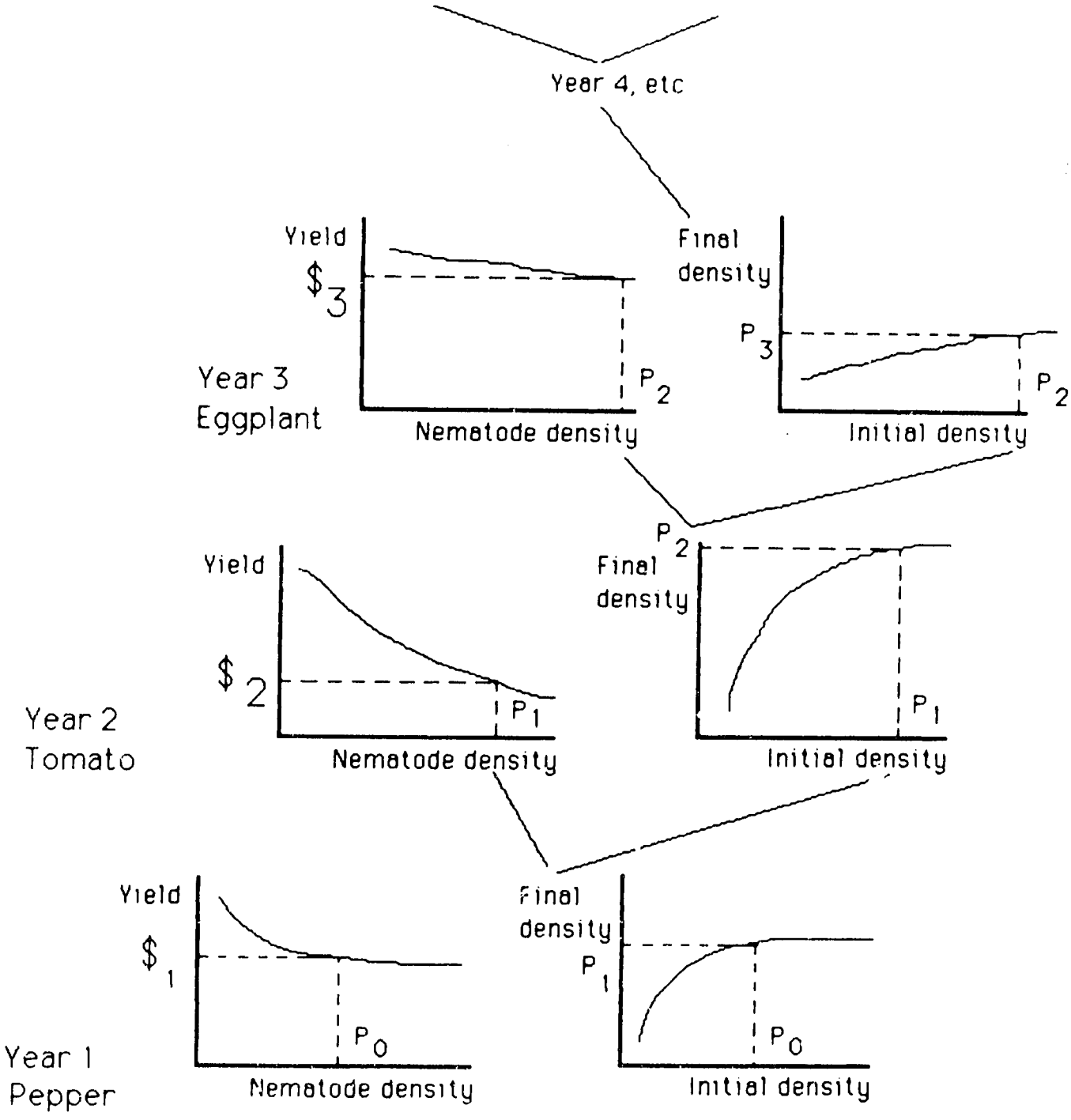

Fig. 12. Linking of characteristic nematode-host relationships in simulation of a cropping sequence. A beginning initial nematode density (P_0) is used to predict end-of-season density (P_1) and yield of the crop for the first year. End-of-season density is then used as beginning density (after adjustment for between-crop survival) for the next crop, to predict end-of-season population (P_2) and crop yield for the second year. This process continues iteratively as many seasons (crops) as desired.

formulating a mathematical representation of all the constraints, demands and desired objectives in a production system and deriving the optimal solution, or most profitable course of action. All possible sequences can be considered simultaneously by the computer.

The accuracy with which a linear programming system represents its intended real-world system depends entirely on how well the mathematical relationships representing constraints and objectives are formulated. Information on fixed and variable costs of crop production must be accurate, as well as estimates of the additional costs incurred by the use of an optional management practice. Estimated future prices for each crop must be relatively accurate, and cultural variables, such as the desirability of each crop for local consumption, must be appropriately quantified. Suitable estimation of these variables will be contingent on the experience, knowledge, and resources of the local researcher. After these estimates are provided, the linear optimization scheme will be formulated with assistance from the CNRCP cropping systems specialist, or with the cooperation of local personnel with experience in this area of mathematics.

Validation is essential in any modelling process. The crop sequencing models will be validated by the monitoring of selected rotations in the experimental plots over several growing seasons. By comparison of projected to actual crop losses and nematode population dynamics in the plots, a measure of stochastic variation in the model will be derived. The model will be adjusted and improved with each year's data, but there will likely be considerable stochastic error in the projections. This error will be reported and included in the cropping system recommendations as a measure of the reliability of the system. Reliability may be low in the early stages of the process until other pests and edaphic parameters are brought into the models, but the results will still represent the best recommendation possible based on existing information.

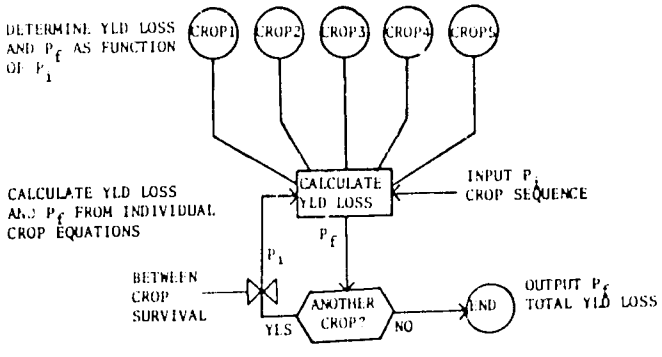

Fig. 13. Flow-chart of computer algorithm to simulate by step-wise calculations a selected crop sequence.

TECHNOLOGY TRANSFER

Final delivery of the results of this research to farmers will be in the form of extension-type publications, pamphlets listing recommended cropping sequences for particular nematode hazards, and an assay-based nematode advisory recommendation form (Fig. 14). Where feasible, the assay-based recommendation form would be the desired method of transfer since it offers specific recommendations for individual farmer problems. On this form, the farmer will be given a listing of important plant-parasitic species found in his field, along with an indication of crop hazards associated with detected population densities. The assay form will also contain a list of recommended management sequences, including the use of nematicides or resistant cultivars if appropriate, with projected net profits for each sequence. The results of the worst possible cropping sequence will also be displayed to indicate the possible losses from poor management practices. The computer programs necessary to produce the recommendation forms will be provided to cooperating scientists with adequate computer or microcomputer facilities. The use of this system is dependent on availability of nematode assay services for farmers. Where assays are not available, extension publications and pamphlets will be used to provide general cropping systems recommendations for limiting losses due to important nematode pests.

CONCLUDING REMARKS

The cropping systems experimental design is relatively simple to implement. Field plots are located in nematode-infested areas, and three variables are measured in each plot. These three variables, initial nematode density, end-of-season density, and yield, then are used in a complex data analysis scheme to produce an optimizing model of the cropping system. As part of this analysis, quantitative crop-nematode relationships are derived which will be of value in other areas of research and in crop-loss assessment programs. The data-collection phase of the research is lengthy and labor intensive, but the data analysis will be relatively fast and less arduous with assistance from the CNRCP cropping systems specialist. From a well-structured analysis of only three variables collected from nematode-infested field plots, several important benefits are anticipated:

1. Development of the best possible cropping system for farmers to control losses due to plant-parasitic nematodes,
2. Quantification of important crop-nematode relationships which are necessary for implementation of nematode management-advisory

Country	Cooperating scientist
Grower	

Nematode assay results		
Nematode species	Number of nematodes [a]	Crop loss hazard level
Meloidogyne spp	**10547**	**high**
Paratrichodorus	**459**	**low**
Heterodera	**2078**	**low**
Scutellonema	**994**	**medium**

Cropping system recomendations					
Crop					Expected net profit [b]
one	two	three	four	five	
eggplant	**eggplant**	**tomato**	**pepper**	**tomato** [c]	**1543**
barley	**tomato**	**pepper**	**tomato** [d]	**eggplant**	**1427**
tomato [d]	**pepper**	**eggplant**	**barley**	**tomato**	**1298**
Worst projected cropping sequence					
tomato	**tomato**	**tomato**	**tomato**	**tomato**	**447**

Notes

[a] number of nematodes per 500 cm^3 soil.
[b] net profit projected in local currency.
[c] use nematicide with recommended crop.
[d] use resistant cultivar

Fig. 14. Example of a nematode assay/crop management recommendation form, resulting from simulation and optimization techniques used in cropping systems research. Form includes nematode counts, crop hazard levels, and specific management recommendations for the farmer to consider.

services and for accurate assessment of crop losses due to these pests,

3. Increased profits and economic stability for farmers following recommended long-term cropping sequences, and
4. Increase in available food and fiber for within-country consumption or export.

All of these benefits can be derived from an improved deployment of existing crop management techniques. The improvement will result from optimized decision-making based on a systematic analysis of field research data. Ideally, the cropping system should be optimized with respect to other pests, but the information is not yet available to construct appropriate models. Nematode cropping systems research is only one component of a comprehensive farming systems analysis (Fig. 15). However, after analysis of the nematode component of the pest complex with a well-structured, standardized research design, other pests can be added to the system as data becomes available from similar research programs. In this way, a comprehensive decision tool can be developed over a number of years by different researchers. The ultimate goal of providing farmers with detailed recommendations as to the best possible management tactics, considering all production constraints simultaneously, will then be realizable.

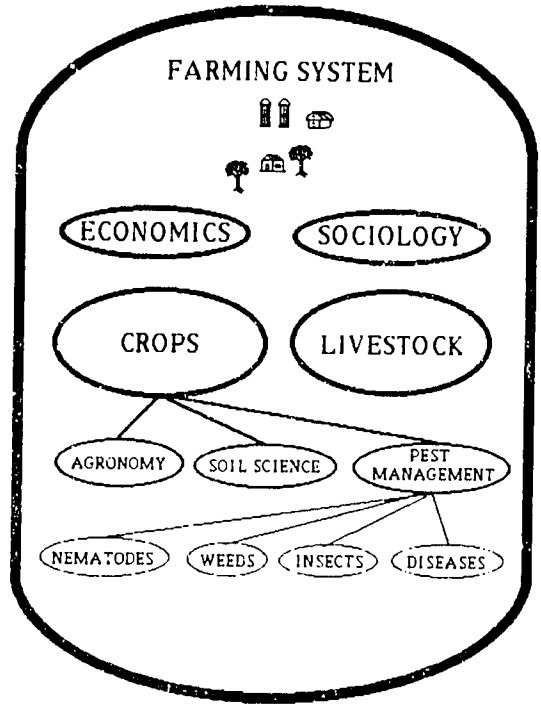

Fig. 15. Schematic diagram of interrelated components of a comprehensive farming system. Methods presented in this guide are for analysis of the nematode-pest component separately, but the analysis will be done in such a way that other components can be included in the design as additional information is obtained.

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