

Interactions Between Nematodes and Other Factors on Plants

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Abstract: The distinction between qualitative and quantitative interactions is stressed as it helps to elucidate differences between two current definitions of the term synergism and how multifactorial experiments should be designed, analysed statistically, and their results interpreted. Factorial design and analyses are strongly advocated so that interactions can be detected. It is suggested that interactions involving nematodes are common in nature and should be included, where appropriate, in hypotheses. Methods for testing the hypothesis that environment influences tolerance are suggested. *Key words:* interactions, factorial ANOVA, response surfaces, synergism, antagonism, tolerance.

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In nature, plant diseases are the result of several determinants (16,34,38), some of which are living organisms and others are termed abiotic factors. A further complexity, again a feature of the real world, arises when the determinants influence each other in their effects on the plant; that is, they interact. Consequently, the contribution to disease of the various determinants may not be additive. It is this aspect with which this paper is concerned and, because the interaction is essentially statistical in nature, much emphasis is placed on the interactions of these factors.

Interactions involving nematodes may be important for several reasons. First, there is sufficient information in the plant pathology literature to indicate that they may contribute substantially to variability in crop growth (38). Second, interactions as a

feature of complexity in crop systems need to be understood if such complexity is to be reduced to a level where controlled experiments can be devised to test hypotheses involving nematodes. Third, it is possible that some catastrophic crop losses involving nematodes are not just the consequence of the coincidental occurrence of several determinants but of interactions between them that exacerbate the effects. Finally, the word "interaction" has been given several meanings in the nematology literature, often in a descriptive and imprecise way.

This paper attempts to describe, in a statistical sense, quantitative interactions where plant response is determined by the influence of two or more factors which act at different levels. Such interactions are distinguished from qualitative interactions where the presence or absence of a particular plant response is measured and where one of the factors is a nonpathogen. This distinction between quantitative and quali-

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tative interactions is important because, as will be shown later, it has resulted in different perceptions of such concepts as synergism and how multifactorial experiments should be designed and their results interpreted.

THE STATISTICS OF INTERACTIONS

When the effects of two or more determinants on plant growth are additive, the hypothesis can be expressed in terms of the equation:

$$Y = b_1x_1 + b_2x_2 + \dots + b_nx_n + e$$

where Y is some measure of plant growth, b_1, b_2, b_n are constants, $x_1, x_2 - x_n$ are the independent variables (e.g. numbers of nematodes, temperature, soil water, etc.), and e is the error term.

When two independent variables interact, a further term (x_1x_2) has to be included. The equation now reads:

$$Y = b_1x_1 + b_2x_2 + b_3(x_1x_2) + \dots + b_nx_n + e$$

Interactions may appear in so-called synoptic studies (30, 34) of crop growth where the influence of variables and their interrelationships on crops are studied and in experiments where variables are manipulated to give several combinations of treatments. In both events, the measured variance in the dependent variable Y , usually the plant, is divided into portions which serve as unbiased estimates of the variation due to the different sources such as environmental factors or experimental treatments. McSorley and Waddill (21) used stepwise multiple regression analysis to partition yield loss on yellow squash between nematodes and insects. Yield losses, in weight, of small fruit to nematode and insect pests together were estimated at 23.4 and 30.4%, respectively. In this multiple-pest system, interactions on yield occurred between *Rotylenchulus reniformis* (Linford and Oliveira) and the insect *Diaphantia hyalinata* (L).

An analysis of variance allows the contributions of factors to variance to be assessed through calculation of sums of squares, variance ratios, and the F test. Multiple-range tests can then be used to identify significant differences between means of treatments. Although it is usually

possible to arrive at a significant F statistic, provided enough replicates are used, the important part of the analysis of variance is not the F test but the relative sizes of the mean squares that indicate the relative contributions to variance of the components in the analysis of variance model.

The assumptions on which the analysis of variance are based sometimes require data to be transformed. Such transformations may eliminate what at first sight appeared to be significant interactions, and the units, in which the dependent variable (Y) is expressed, may also determine whether an interaction term should be included in the model. For example, an interaction between independent variables may occur when the dependent variable is expressed as plant growth increment ($X_T - X_0$) but disappear if relative growth ($(X_T - X_0)/X_0$) is used, where X_T and X_0 are plant weight at times T and O . Required transformations must be met and the units of plant growth must satisfy the physiological nature of the plant response. If interactions still make a statistically significant and a substantial contribution to observed variance in plant growth, then they assume an important part of the hypothesis. It is more useful, for example, to consider two interacting factors together rather than separately (35). For example, Roberts and van Gundy (28) studied the joint effects of temperature and populations of *Meloidogyne javanica* (Trent) Chitwood on tillering in wheat. Temperature alone had a statistically significant effect and nematode populations had no significant effect, but the interaction between *M. javanica* and temperature was highly significant. Thus, in this experiment it is unrealistic to consider the influence of the nematode on tillering without referring to temperature; the two factors are inextricably linked.

Experiments that indicate significant interactions seldom indicate the mechanism of the interaction. Further hypotheses and quite different experiments, usually of a physiological nature, are required.

In some ways interactions are a nuisance because they prevent a simple explanation by a purely additive model, and if interactions are not the concern of the experimenter, there is usually a transformation

that removes them. However, interactions arising from biological events and involving nematodes do occur and there is no escaping the fact that the real world of the crop and the factors that determine its growth and yield contain such complexities (13).

THE DESIGN OF EXPERIMENTS

There are numerous descriptions of experiments in the literature showing the effects on a plant of a nematode and some other factor (another nematode species, a fungus, an environmental factor, etc.) where the two act alone, together, and with neither present. Multiple-range tests have been used to indicate whether plant responses to these four types of treatment are significantly different (17,18,20,24). Interaction terms are not mentioned and presumably were not obtained. Although no errors or misinterpretations are evident, more useful information about interactions could nevertheless have been obtained from the data. For example, there are numerous statements that the combined effect of a nematode and some other factor is greater than the effect of *either alone*, but such conclusions fail to answer the more important question whether nematode and factor together are greater than the *sum of the individual effects*. Simple arithmetic soon gives an answer, of course, but except where differences between treatments are so large as not to require statistics, there is no indication whether the differences are likely to be real or due to chance.

Such difficulties arise through inappropriate analysis and design. Thus, even in experiments where presence and absence of a nematode and some other factor are used to give four treatments, it is possible by expressing the results in a two-way table to calculate by a two-way analysis of variance the interaction term between nematode and the other factors although, as for each main effect, there is only one degree of freedom (31). As only two levels for the nematode and other factor were used and one of these was zero, it is invalid to generalize from such an experiment and conclude that an interaction does not occur. If other levels of the variables had been used, a significant interaction might well

have been found (8). Thus, the use of a range of nematode populations or levels of variables is desirable (29). It enables a series of combinations to be used, and a one-way analysis of variance and a multiple-range test, to assess differences between treatments, give more information than the presence and absence type of experiments. But much more information can be achieved with a factorial experiment (8) using a two-way analysis of variance. For example, it might be possible to say whether the effects of a nematode and another factor were acting independently or whether either was reinforcing (synergism) or inhibiting (antagonism) the other.

More than two independent variables can be used and higher order interaction terms derived (12,32). However, the contribution that interactions make to explaining the response in the dependent variable (the plant) seems to decrease as the order of interaction increases (13). Although there is little evidence for this statement, there is no doubt that the interpretation of second-order interactions (involving three variables) is difficult. Furthermore, where treatments are applied at several levels, in a factorial design, there is a practical limit to the number of variables that can be used. Thus, three variables, each at five levels and with fourfold replication, requires 4×5^3 or 500 containers or plots. Even using confounding procedures to reduce numbers of experimental units, the scale of such experiments is daunting; hence it is probably valid to conclude that each interaction experiment should be confined to no more than three variables; i.e., a nematode and two other factors.

Three-dimensional graphs to show the effects of two independent variables on plant growth allow response surfaces to be portrayed (14,15). Such surfaces often provide insight into the nature of interactions by indicating, for example, whether there is curvilinearity in the response. "Presence and absence," or 2^n factorial experiments (n = number of treatments), provide little information on response surfaces (3), but they are useful in an exploratory approach to a problem where only qualitative conclusions are sought or where variables cannot be used at various levels as with viruses.

SYNERGISM AND ANTAGONISM

Dickinson (7) has discussed the introduction of the word "synergism" into plant pathology along with its definition as "an association of two or more organisms acting at one time and effecting a change that one only is not able to make." Such a definition is essentially qualitative and seems more attuned to studies where one of the two interacting factors is nonpathogenic and in some way increases the disruptive effects of the pathogen on the plant. Such responses are described in several of the 2ⁿ type of experiments in the nematology literature. For example, Mitchell and Powell (22) showed that *Fusarium oxysporum* f.sp. *vasinfectum* (Atk.) Snyder & Hans. alone caused 20% of plants of the *Fusarium* wilt-susceptible cultivar of cotton, Deltapine Smoothleaf, to wilt. *Pratylenchus brachyurus* (Godfrey) Filipjev and Stekhoven on its own caused no wilting but in combination with the fungus caused 40% of the plants to wilt. No wilting occurred in the absence of both organisms. The term "synergisms" was not used in this study.

A contrary view has been expressed by Powell (27) who, quoting Agrios (1), defines synergism as "the concurrent or sequential pathogenesis of a host plant by two (or more) pathogens in which the combined effects of the pathogens are greater than the sum of the effects of each pathogen alone."

The two definitions are quite different; that described by Dickinson (7) is qualitative and restricted in its application, whereas that given by Powell (27) is quantitative and more useful in nematology where interacting factors need to be used at several levels if their influence on the plant is to be understood. Furthermore, the term "synergism" is sometimes used in statistics to denote a positive interaction; i.e., the sum of treatment effects is not simply additive (25).

Thus, it is concluded that the term "synergism" should be confined to those plant responses where a positive interaction has been found. Similarly "antagonism" should denote a negative statistical interaction because the combined effect of the factors is less than the sum of the effects of

each factor alone. It may be wise to err on the cautious side and avoid the terms altogether; they seem unnecessary where interactions are not involved and where they are, positive or negative interactions are adequate descriptions of the events.

IMPORTANCE OF INTERACTIONS IN NATURE

That plants are frequently subjected to insults from nematodes in association with other factors is well documented (10,26), but whether subsequent plant response is merely caused by a sum of the individual effects of whether interactions occur is less well understood.

Sometimes nematodes alone have little effect on the plant, but in combination with another organism (8) or an abiotic factor (12,23,28,32), an interaction occurs resulting in a marked effect on the plant. In such cases the effect of the nematode on the plant can only be described in terms of the second factor.

In addition to those already mentioned, experiments have been described in which significant interaction terms have been found (6,8,11,21,36,37). Others did not detect interactions (2,5,9,19,31). Although there are probably other examples, what evidence there is does not contradict the hypothesis that interactions between nematodes and other organism or abiotic factors are common. Hence it may be wise to include them in any hypothesis where the influence of two or more factors on plants is being studied. To omit or deny their existence at this stage may lead to misinterpretations of field data.

INFLUENCE OF ENVIRONMENT ON TOLERANCE

A further question can now be asked: "Where statistically significant interactions involving nematodes occur, is it valid to conclude that environment influences the plant's ability to tolerate disruption by the nematode?" Put more succinctly, "Does environment influence tolerance?" If tolerance is defined as the ability of a plant to maintain growth in spite of damage and is measured in terms of some plant property such as relative growth or yield, then the answer

is "yes"—provided further experiments of a physiological nature support the hypothesis that the environment influenced, in some way, the plant's ability to heal wounds, regenerate tissues replace roots, etc. In other words, that environment influenced the mechanism that confers tolerance in the plant. However, interaction experiments of the kind referred to so far usually provide little information on the physiological mechanisms behind the response. Thus, in the experiments of Edongali and Ferris (11), damage to tomatoes by *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 was generally more evident at higher salinity levels where conditions were unfavorable for plant growth. However, such conditions were also unfavorable for the nematode, resulting in lower final population densities. The authors suggest that salts may function as stimulant or depressant factors to various physiological functions in the plant, especially when combined with other stressful agents, such as nematodes; at lower concentrations they may stimulate growth. Other physiological hypotheses could be proposed, but all would require further experiments to test them.

Seinhorst's (29) view that there is little evidence that external conditions influence tolerance is probably correct, and earlier statements (4,33,34) stressing the importance of this relationship are probably premature. Nevertheless, the hypothesis that environment may influence the tolerance of plants to nematode attack is useful and, providing a definition of tolerance can be agreed, it is certainly testable. For example, in my opinion the significant interactions between nematodes and salt concentration on tomato growth as described by Edongali and Ferris (11) support the hypothesis.

CONCLUSIONS

Interactions involving nematodes probably make a substantial contribution to variation in crop yields. Appropriate experimental design and statistical analyses are necessary if interactions are to be detected and in considering these aspects, the following conclusions emerge:

1. Plant diseases are a consequence of the effects of several determinants, including nematodes, which may interact with

each other in ways that can be measured statistically.

2. Interactions between nematodes and other factors occur when the combined effect of both in the same plant is not additive.
3. If the value of the mean square for an interaction term in an analysis of variance is substantial as well as having a significant F value, then it is unrealistic to consider the influence on the plant of the nematode alone. Nematode and other factors are inextricably linked and must be considered together.
4. Interactions are a feature of the real world and reflect one aspect of complexity in the crop ecosystem.
5. "Presence and Absence," or 2ⁿ, experiments are useful in exploratory approaches to a problem or where a variable, such as a virus, cannot be used at different levels. In such experiments a factorial analysis of variance should be used to determine if there is a significant interaction term (one D.F.). Such interactions are essentially qualitative and provide limited information. Thus, absence of an interaction does not necessarily indicate that one would not have been found if different levels of the variables had been used.
6. Where practicable, experiments to study the influence of nematodes and another factor on a plant should use several levels of each in a factorial design. After necessary transformations the data should be analysed by analysis of variance and interaction terms derived.
7. It may be impractical to use more than three variables at a time in an interaction experiment. Second and higher order interactions are difficult to interpret (i.e., lead to clear testable hypotheses), may contribute little extra knowledge, and may be cumbersome, although the use of confounding procedures can go a long way to meet this problem.
8. Response surfaces may assist in the interpretation of interactions.
9. In spite of earlier more restrictive definitions, synergism and antagonism (interference) are best considered as interactions. In essence, the terms imply

- that the combined effect of a nematode and one or more biotic or abiotic factors are respectively greater or lesser than the sum of the effects of the individual factors.
10. Interactions occur frequently in nature, and there is no reason to expect that they will be any less common where nematodes are involved. Hence, it is necessary to include them, where appropriate, in hypotheses.
 11. Depending on the definition of tolerance, it is possible that environment influences this defensive property of the plant. To test the hypothesis requires experiments that show unequivocally that an interaction occurs between a nematode and some other environmental factor.
 12. The interpretation of factorial experiments with interactions is difficult as there are usually several explanations. Thus, interaction hypotheses require testing at the plant physiological level.

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