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REVIEW ARTICLE

Uncertainty and sensitivity analysis for models used in pest risk analysis

D. Makowski

Summary Quantitative models have several advantages compared to qualitative methods for pest risk analysis; quantitative models do not require the definition of categorical ratings and can be used to compute numerical probabilities of entry and establishment, and to quantify spread and impact. However, quantitative models include several sources of uncertainty that need to be taken into account by risk assessors. In this paper, we review the four main sources of uncertainty in models used for pest risk analysis, namely input variables, parameter values estimated from expert knowledge, parameter values estimated from data and equations. We discuss the practical interest of uncertainty and sensitivity analysis for pest risk assessors. Uncertainty analysis consists in describing the different uncertain elements of a model, and deducing an uncertainty distribution for each output variable rather than a single value. The aim of sensitivity analysis is to determine how sensitive the output of a model is with respect to elements of the model which are subject to uncertainty. Uncertainty analysis typically comprises three main steps: i) definition of uncertainty ranges and/or of probability distributions for uncertain model elements, ii) generation of values of the uncertain model elements, iii) model output computation and description of model output distribution. Sensitivity analysis includes another step to compute sensitivity indices (step iv). When several model equations are available for predicting a given quantity of interest, a further step is to analyse uncertainty about model equations using specific techniques. Several methods were illustrated in a case study on *Sclerotinia sclerotiorum*. Results showed that a moderate uncertainty on parameter values can induce a large uncertainty on model output.

Additional keywords: biological invasion, model prediction, model selection

Introduction

Risk analysis includes a series of steps from initiation, through qualitative or quantitative assessments of risk, to the resultant management decisions. It also includes communications with stakeholders throughout the process. In plant health, Pest Risk Analysis (PRA) consists of the assessment of the probabilities of entry and establishment of an invasive species, the magnitude of the impact resulting from an invasive species, and of management options. Both quantitative and qualitative methods have been used for PRA. Qualitative methods based on scoring systems are a primary choice for assessing risk in plant health but, in several cases, quantitative models have been developed and used in PRA (e.g. Stansbury *et al*., 2002; Peterson *et al*., 2009).

Qualitative methods for PRA are based on categorical ratings (e.g. low, moderate, high) and the use of such ratings may lead to problems of consistency due to inaccurate definitions of ratings. Qualitative methods also make the computation of an overall risk level difficult because categorical ratings can be combined using many different techniques which may lead to different conclusions (Holt, 2006). In addition, the performance of qualitative PRA methods depends on the technique chosen for combining cat-

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egorical rating (e.g. sum, multiplication) as shown by Makowski and Mittinty (2010).

The use of quantitative models has several advantages compared to qualitative methods for pest risk analysis. Quantitative models do not require the definition of categorical ratings and can be used to compute numerical probabilities of entry and establishment, and to quantify spread and impact (EFSA, 2008a). Quantitative models can also be used to assess and select qualitative scoring systems for PRA (Makowski and Mittinty, 2010).

Quantitative models are generally not used to perform full PRA, but rather to estimate some elements of PRA like probability of entry, probability of establishment, spread, or impact. Several probabilistic models have been developed to predict probability of entry of pests through imported commodities (e.g. Roberts *et al*., 1998). A great diversity of models has been used to assess the risk of establishment of pest from bioclimatic variables: statistical models (e.g. Roura-Pascual *et al*., 2009), models based on machine learning techniques (e.g. Phillips *et al*., 2006), models taking into account the ecological processes involved in biological invasion like Climex (Young *et al*., 1999) and NAPPFAST (Magarey *et al*., 2007). Epidemiological models have been developed to assess risk of spread and impact (Stansbury *et al*., 2002).

These models are powerful tools, but they include several sources of uncertainty that need to be taken into account by risk assessors and communicated to decision makers. In this paper, we review the main sources of uncertainty in models used for PRA, and discuss the practical interest of uncertainty and sensitivity analysis for pest risk assessors. The paper is organized as follows: Sources of uncertainty in models used for PRA are presented in section 1. The objectives of uncertainty and sensitivity analysis are presented in section 2 and the main steps of these two types of analysis are described in section 3. Finally, a case study is presented in section 4.

1. Origins of uncertainty in models used for pest risk analysis

Models used for PRA can include up to four sources of uncertainty, namely input variables, parameter values estimated from expert knowledge, parameter values estimated from data, and equations. Input variables correspond to variables whose values vary between sites and/or year and can be measured. Climatic variables, such as temperature annual range or annual precipitation, are typical examples of input variables. Climatic variables can be measured from weather stations, but their values are often imperfectly known due to error of measurement or due to the absence of weather station in the sites of interest. Climate change can also increase uncertainty (Araujo and New, 2006; EFSA, 2008a).

Parameters correspond to model components whose values cannot be directly measured but need to be estimated from expert knowledge, from data, and from both expert knowledge and data. When parameters are estimated from expert knowledge, the accuracy of the estimates depends on expert bias and on the method used for expert knowledge elicitation (O'Leary *et al*., 2008). When parameters are estimated from data, the accuracy of the parameter estimates depends on the estimation technique and on the quality of the dataset. Consider, for example, models used for mapping invasive species distribution from bioclimatic variables. These models include parameters that need to be estimated from a set of species presence records and, if possible, from a set of species absence data (Vaclavik and Meentemeyer, 2009). It was shown that the performances of these models were related to the size of the datasets and to the reliability of presence and absence data (Wisz *et al*., 2008; Vaclavik and Meentemeyer, 2009; Giovanelli *et al*., 2010).

Model equation is another source of uncertainty. Several alternative models may be available for a given practical problem, especially for predicting invasive species distribution (Roura-Pascual *et al*., 2009). In such

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cases, the traditional approach is to take a model selection process to find the best model from which one makes practical applications. Several criteria have been proposed for selecting models using a test dataset (e.g. Smith *et al*., 1999; Townsend Peterson *et al*., 2008). However, potential problems have been recognized by statisticians. An important concern is that the uncertainty in model selection is basically ignored once a final model is found (Chatfield, 1995; Draper, 1995). Final estimation, interpretation of the parameter values, and model predictions are generally based on the selected model only. In some cases, the instability of the result of a selection process is high; Yuan and Yang (2005) showed that, when the model errors are large, a selection process is likely to lead to a completely different selected model when a slightly different dataset is used. The selected model may also depend on the criterion used for model selection and, as shown by Townsend Peterson *et al*. (2008) and by Lobo *et al*. (2008), there is no consensus in the scientific community on the best criterion for selecting models for predicting biological invasion. For all these reasons, it is never sure that the selected model is the most appropriate one for practical applications.

2. Objectives of uncertainty and sensitivity analysis

Uncertainty analysis consists in evaluating quantitatively uncertainty in model components (input variables, parameters, equations) for a given situation, and deducing an uncertainty distribution for each output variable rather than a single value (Vose, 2000; Monod *et al*., 2006). It can be used, for instance, to compute the probability of an output variable of interest (e.g. number of spores entering in a given area) to exceed some threshold (e.g. Peterson *et al*., 2009). Uncertainty analysis is a key component of model-based risk analysis because it provides risk assessors and decision makers with information about the accuracy of model outputs. In pest risk analysis, uncertainty analysis was used by several authors to estimate probability of entry and establishment (Stansbury *et al*., 2002; Peterson *et al*., 2009; Yen *et al*., 2010), spread of invasive species (Koch *et al*., 2009), and to assess efficiency of management options (Yen *et al*., 2010).

The aim of sensitivity analysis (SA) is to determine how sensitive the output of a model is with respect to elements of the model which are subject to uncertainty. For dynamic models, sensitivity analysis is closely related to the study of error propagation. As in SA, input variables and parameters have the same role, uncertain input variables and parameters will be further denoted as uncertain factors. Two types of sensitivity analysis are usually distinguished, *local* sensitivity analysis and *global* sensitivity analysis (Saltelli *et al*., 2000). Local SA focuses on the local impact of uncertain factors on model outputs and is carried out by computing partial derivatives of the output variables with respect to the input factors. With this kind of methods, the uncertain factors are allowed to vary within small intervals around nominal values, but these intervals are not related to the uncertainty in the factor values. Contrary to local SA, global SA considers the full domain of uncertainty of the uncertain model factors. In global SA, the uncertain factors are allowed to vary within their whole ranges of variation.

Sensitivity analysis may have various objectives, such as:

- to study relationships between model outputs and model inputs;
- to identify which input factors have a small or a large influence on the output;
- to identify which input factors need to be estimated or measured more accurately;
- to detect and quantify interaction effects between input factors;
- to determine possible simplification of the model;

In pest risk analysis, sensitivity analysis techniques were used to study the sensitivity of spatial model predictions to input factor values (Koch *et al*., 2009) and to data

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used for parameter estimation (Vaclavik and Meentemeyer, 2009). Sensitivity was also used to identify the most important factors influencing the predicted efficiencies of different management options (Stansbury *et al*., 2002; Roura-Pascual *et al*., 2010).

3. Main steps for uncertainty and sensitivity analysis

Uncertainty analysis typically comprises three main steps: (i) definition of uncertainty ranges and/or of probability distributions for uncertain model input factors, (ii) generation of values for the uncertain input factors, (iii) model output computation and description of model output distribution. Sensitivity analysis includes another step to compute sensitivity indices (step iv). Finally, when several model equations are available for predicting a given quantity of interest, a further step is to analyse uncertainty about model equations using specific techniques. All these steps are detailed below.

3.1. Step (i). Uncertainty ranges and probability distributions for uncertain input factors

Uncertainty in an input factor can be described in different ways. It is often described by the most likely factor value plus or minus a given percentage (e.g. Koch *et al*., 2009) or it is specified through a discrete or continuous probability distribution over a range of possible values. Among probability distributions, the uniform distribution, which gives equal weight to each value within the uncertainty range, is commonly used in uncertainty and sensitivity analysis when the main objective is to understand model behaviour.

More flexible probability distributions are sometimes needed to represent the input uncertainty. When the input corresponds to a discrete variable (e.g. number of imported consignments, number of successful entries, etc.), discrete probability distribution (e.g. Poisson distribution) is often appropriate (e.g. Yen *et al*., 2010). Among continuous distributions, the well-known Gaussian distribution is often convenient since it requires only the specification of a mean value and a standard deviation. It is often replaced by the truncated Gaussian distribution, triangular, or by beta distributions, which give upper and lower bounds to the possible values (e.g. Peterson *et al*., 2009; Yen *et al*., 2010). When the distribution should be asymmetric, for example when input factors are likely to be near zero, lognormal, triangular, or beta distributions offer a large range of possibilities (e.g. Peterson *et al*., 2009).

Probability distributions can be derived from expert knowledge and/or from experimental data. Bayesian statistics now offer a variety of methods and algorithms to derive probability distributions by combining expert knowledge and data (e.g. Gelman *et al*., 2004).

3.2. Step (ii). Generation of values of uncertain factors

Monte Carlo sampling is a popular method for generating representative samples from uncertain factor distributions. In Monte Carlo sampling, the samples are drawn independently, and this approach provides unbiased estimates of the expectation and variance of each output variable. Other alternative sampling techniques like Latin Hypercube can be used. It is also possible to generate combinations of values of uncertain factors by using experimental designs like, for example, complete factorial designs. This approach was used by EFSA (2008b) to combine minimum, maximum, and most likely values of several uncertain input factors.

3.3. Step (iii). Model output computation and description of the model output distribution

This step may be difficult to carry out when computation of model output is time-consuming. With some very complex models, the number of samples generated at the previous step must be set equal to a small value due to computation time constraint.

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On the contrary, computation is straightforward for models that are less complex and less computationally intensive.

Output values can be presented in different ways. In general, it is not appropriate to present all the computed model outputs because the number of computed values is generally very high (i.e. several thousands). The recommended approach is to summarize the output distributions by calculating several key parameters such as mean, median, standard deviation, coefficient of variation, several extreme percentiles (1%, 5%, 10%, 90%, 95%, 99%). It is also useful to show some graphical presentations of the computed model outputs, like histograms and cumulative probability distributions. All these techniques have been applied in several quantitative risk assessments (e.g. Koch *et al*., 2009; Peterson *et al*., 2009). When the model includes several output variables, it is useful to analyse the relationships between these variables by drawing scatter plots or by computing correlation coefficients.

3.4. Step (iv). Computation of sensitivity indices

Sensitivity of model output to an uncertain factor is commonly studied by using simple graphical presentation of model outputs *versus* model inputs (e.g. Koch *et al*., 2009; Giovanelli *et al*., 2010). This approach is useful but not sufficient to assess and compare the influence of the different input factors in a quantitative way. It is recommended to compute sensitivity indices for all the uncertain factors in order to rank these factors according to their influence on the outputs.

A sensitivity index is a measure of the influence of an uncertain factor on a model output variable. Factors whose values have a strong effect on the model are characterized by high sensitivity indices. Non-influential factors are characterized by low sensitivity indices. Sensitivity indices can thus be used to rank uncertain factors and identify those which should be measured or estimated more accurately.

A great diversity of sensitivity indices has been proposed (e.g. Saltelli *et al*., 2000). In local SA, sensitivity indices are based on derivative calculation and correspond to the slopes of the model output in the input factor space at a given set of values. In global SA, sensitivity indices can be computed using a variety of techniques like ANOVA, correlation between input factors and model outputs, Fourier series, Monte Carlo simulations, etc. (Saltelli *et al*., 2000). Sensitivity indices can be computed using statistical software (e.g. the package sensitivity of the statistical software R http://cran.r-project.org/web/packages/sensitivity/index.html) or more specialized software, such as Simlab (http://simlab.jrc.ec.europa.eu/), @Risk, or Crystal ball. Examples of calculation of ANOVA-based sensitivity indices in quantitative pest risk assessment can be found in EFSA (2008b). Examples of correlation-based sensitivity indices are provided in the case study presented at the end of this paper.

3.5. Specific methods for analysing uncertainty in model equations

Many models are now available for estimating risk of entry, establishment, and spread. In some cases, it is difficult to choose the most appropriate model for a given question. For example, five different models were used to map invasive species distribution by Roura-Pascual *et al*. (2009) and these models led to different predictions generating uncertainty about the potential distributional area.

Two approaches have been proposed to deal with this uncertainty, model comparison and model mixing. The latter approach is also called consensual predictions or ensemble forecasting. Model comparison aims at assessing several candidate models in order to select the model with the best predictive performance. Several criteria have been proposed to assess models for predicting invasion (e.g. Smith *et al*., 1999; Townsend Peterson *et al*., 2008) and the most popular criterion is probably the area under the Receiver Operating Characteristic (ROC) curve, which measures the ability of models to discriminate presence and absence locations.

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A limitation of model comparison is that, in some cases, several models show similar performance (e.g. Hernandez *et al*., 2006; Roura-Pascual *et al*., 2009). Another issue is that reliable data are not always available. Model selection is then somewhat arbitrary.

Several statisticians emphasised that, in some cases, it is better to mix all models than to use the single selected model. The basic idea is to use a weighted sum of the individual model predictions instead of the prediction derived from the single 'best' model. Several methods were developed to estimate the weight associated to each model from a training dataset (Buckland *et al.,* 1997; Hoeting *et al.,* 1999; Yang, 2003; Raftery *et al.,* 2005; Yuan and Yang, 2005). These methods can be applied to a great diversity of models, linear, logistic, nonlinear, or dynamic models (Raftery *et al*., 1997; Viallefond *et al.,* 2001; Raftery *et al*., 2005), and statistical packages are now available to implement them. See, for example, the BMA R package available at http://cran.r-project.org/web/packages/BMA/index.html and the MMIX R package available at http://cran.r-project.org/web/packages/MMIX/index.html. Both can be freely downloaded and applied using the R statistical software.

Model-mixing methods can improve the accuracy of model predictions and give more realistic confidence intervals (Chatfield, 1995; Draper, 1995). According to a recent statistical study (Yuan and Yang, 2005), model-mixing is better than selection when the model errors are large. Recently, model-mixing methods have been applied for mapping species distribution (Araujo and New, 2006; Marmion *et al*., 2009) and biological invasion (Roura-Pascual *et al*., 2009). It is likely that this approach will be more frequently applied in the future.

4. Case study

In this section, we present a simple case study to show how uncertainty and sensitivity analysis can be used in practice. We consider the simple generic infection model for foliar fungal plant pathogens defined by Magarey *et al*. (2005):

$$W = \min\left\{W_{\max}, \frac{W_{\min}}{f(T)}\right\}$$

and

$$f(T) = \left(\frac{T_{\max} - T}{T_{\max} - T_{opt}}\right)\left(\frac{T - T_{\min}}{T_{opt} - T_{\min}}\right)^{(T_{opt} - T_{\min})/(T_{\max} - T_{opt})}$$

if $T_{\min} \leq T \leq T_{\max}$ and zero otherwise

where T is the mean temperature during wetness period (°C), W is the wetness duration required to achieve a critical disease intensity (5% disease severity or 20% disease incidence) at temperature T. $T_{\min}$, T_{opt}, $T_{\max}$ are minimum, optimal, and maximum temperatures for infection, respectively, $W_{\min}$ and $W_{\max}$ are minimum and maximum possible wetness duration requirements for critical disease intensity, respectively. This model was used to compute the wetness duration requirement as a function of the temperature for many species and was included in a disease forecast system (Magarey *et al*., 2005; 2007).

$T_{\min}$, T_{opt}, $T_{\max}$, $W_{\min}$ and $W_{\max}$ are five species-dependent parameters whose values were estimated from experimental data and expert knowledge for different foliar pathogens (e.g. Magarey *et al*., 2005; EFSA 2008b). However, for some species, these parameters are uncertain due to the limited availability of data (Magarey *et al*., 2005) and, in such cases, it is important to perform uncertainty and sensitivity analysis.

In this case study, uncertainty and sensitivity analysis techniques were applied to the generic infection model defined above for infection of bean foliage by the fungal pathogen *Sclerotinia sclerotiorum*. All computations were done using the freely available statistical software R (http://cran.r-project.org/). Parameter values reported by Magarey *et al*. (2005) for this pathogen are $T_{\min}$=1°C, T_{opt}=25°C, $T_{\max}$=30°C, $W_{\min}$=48 h and $W_{\max}$=144 h but, according to the authors, there is uncertainty about these values. The response curve of W vs. T obtained with the estimated parameter values is presented in

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Figure 1A.

Uncertainty about parameter values was described here by uniform distributions defined with lower and upper bounds set equal to ± 20% of the estimated parameter values reported by Magarey *et al*. (2005): T_{min}~*Unif*(0.8, 1.2), T_{max}~*Unif*(24, 36), T_{opt}~*Unif* (20, 30), W_{min}~*Unif*(38.4, 57.6), W_{max}~*Unif* (115.2, 172.8). The choice of ±20% was done here in order to study the consequence of a moderate uncertainty (the coefficient of variation of the uniform distribution was equal to 28%) on the model output. Other choices are of course possible.

Ten thousands parameter values were randomly generated from the uniform distributions defined above by Monte Carlo sampling. Due to an overlap of the distributions of T_{opt} and T_{max}, a constraint on parameter values was considered at this step in order to satisfy $T_{max}>T_{opt}$. The 10,000 corresponding responses of *W* vs. *T* were computed and a sample of 20 out of the 10,000 response curves was displayed in Figure 1B for illustration. The distribution of the 10,000 response curves was summarized in Figure 1C by the percentiles 1%, 10%, 50% (median), 90%, 99%. The results showed that the uncertainty was more important when the temperature during the wetness period *T* was close to 25°C i.e. the estimated optimal temperature for the fungus (Figure 1C). The distribution of *W* obtained for *T*=25°C was skewed (Figure 1D); the median was equal to

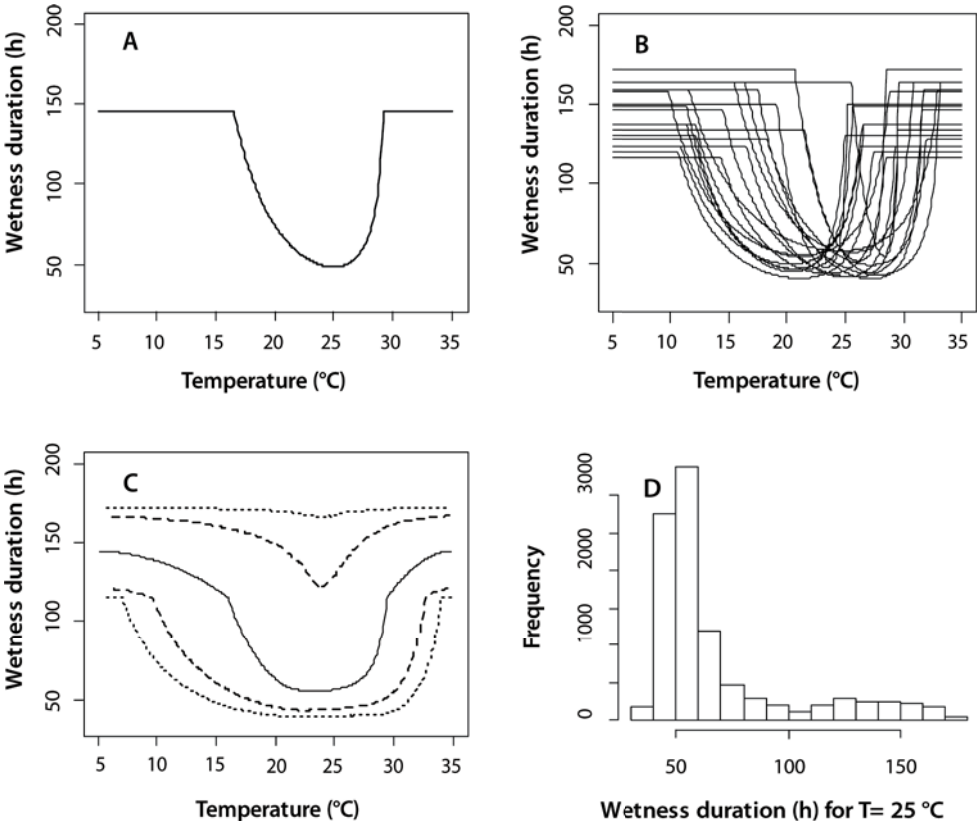

Figure 1. Predicted wetness duration requirements for infection of bean foliage by *Sclerotinia sclerotiorum.* A: Predictions obtained with the parameter values reported by Magarey *et al.* (2005). B: Sample of 20 response curves generated by Monte Carlo simulation. C: Percentiles 1%, 10%, 50%, 90% and 99% of the 10,000 simulated wetness duration requirements in function of the temperature. D: Distribution of the 10,000 simulated wetness duration requirements for *T*=25°C.

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56 h, the 10% percentile was equal to 43.9 h and the 90% percentile was equal 128.7 h. The high coefficient of variation of the distribution of *W* (47%) and the large difference between the 10% and 90% percentiles showed that a moderate uncertainty on parameter values (±20% around the estimated values) can induce a large uncertainty about wetness duration requirement for infection.

In order to identify the main sources of uncertainty, sensitivity indices were computed for the five model parameters for several temperatures *T*. Sensitivity of the model output *W* to parameter values were measured by calculating correlations between *W* and parameter values using the 10,000 Monte Carlo simulations. Results are shown in Figure 2 for all parameters in function of *T*. A correlation close to +1 or -1 indicates a strong influence of the parameter on the model output. A correlation close to zero indicates that the parameter is not influential. More sophisticated sensitivity indices could had been computed (Saltelli *et al*., 2000), but correlation-based indices were considered here because of their simplicity and intuitive interpretation.

Figure 2 showed that correlation be-

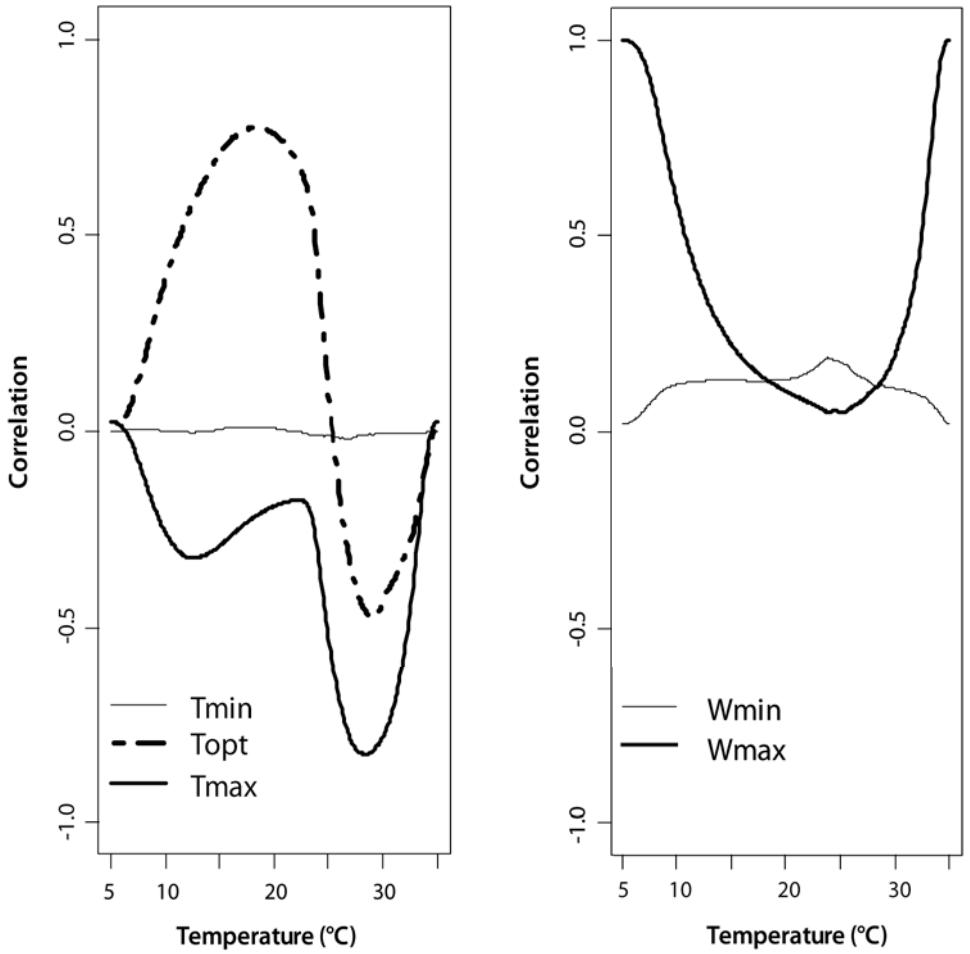

Figure 2. Sensitivity indices for the five model parameters in function of the temperature. Sensitivity indices correspond to correlations between parameter values and wetness duration requirement estimated from 10,000 Monte Carlo simulations.

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tween W and the parameters T_{min} and W_{min} was always close to zero for all temperatures. This result showed that the model output is not sensitive to the values of these two parameters. The parameter T_{opt} had a strong and positive effect on W for temperature in the range 15-20°C, and a strong and negative effect for temperature in the range 27-32°C. Its effect was negligible for extreme temperatures i.e. when T was close to 5°C or to 35°C and when T was close to 25°C. The parameter T_{max} had a negative effect on W, but its effect was negligible for extreme temperatures. When T was close to 5°C or to 35°C, the model output was sensitive to only one parameter: W_{max}. This sensitivity analysis thus reveals that the model output is sensitive to three parameters T_{opt}, T_{max} and W_{max} and that the effect of these parameters is strongly dependent on the temperature.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ανάλυση αβεβαιότητας και ευαισθησίας των μοντέλων που χρησιμοποιούνται στις αναλύσεις επικινδυνότητας επιβλαβών οργανισμών

D. Makowski

Περίληψη Στις Αναλύσεις Επικινδυνότητας Επιβλαβών Οργανισμών τα ποσοτικά μοντέλα έχουν πολλά πλεονεκτήματα σε σχέση με τις ποιοτικές μεθόδους. Τα ποσοτικά μοντέλα δεν απαιτούν τον ορισμό κατηγορικών διαβαθμίσεων και μπορούν να χρησιμοποιηθούν για τον υπολογισμό ποσοτικών πιθανοτήτων εισόδου και εγκατάστασης ενός επιβλαβούς οργανισμού καθώς και για την ποσοτικοποίη-

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ση της διασποράς και τις επιπτώσεις από την εγκατάσταση του οργανισμού σε μια νέα περιοχή. Εντούτοις, τα ποσοτικά μοντέλα περιέχουν πολλές πηγές αβεβαιότητας τις οποίες πρέπει να λάβουν υπόψη τους οι εκτιμητές της επικινδυνότητας. Στην παρούσα εργασία γίνεται ανασκόπηση των τεσσάρων κύριων πηγών αβεβαιότητας των μοντέλων που χρησιμοποιούνται στις Αναλύσεις Επικινδυνότητας, ήτοι των μεταβλητών εισόδου, των τιμών των παραμέτρων που υπολογίζονται με βάση τη γνώση των εμπειρογνωμόνων, των τιμών των παραμέτρων που υπολογίζονται με βάση τα διαθέσιμα δεδομένα και των εξισώσεων. Επίσης συζητείται το ενδιαφέρον που έχει στην πράξη για τους εκτιμητές της επικινδυνότητας η ανάλυση αβεβαιότητας και ευαισθησίας. Η ανάλυση αβεβαιότητας συνίσταται στην περιγραφή των διαφόρων αβέβαιων στοιχείων ενός μοντέλου και στην εξαγωγή συμπεράσματος όσον αφορά στην κατανομή της αβεβαιότητας κατά προτίμηση για κάθε μεταβλητή εισόδου παρά για μια μεμονωμένη τιμή. Σκοπός της ανάλυσης ευαισθησίας είναι να καθορίσει πόσο ευαίσθητο είναι το αποτέλεσμα ενός μοντέλου σε σχέση με τα αβέβαια στοιχεία του μοντέλου. Η ανάλυση αβεβαιότητας τυπικά συνίσταται σε τρία κύρια βήματα: i) ορισμός του εύρους αβεβαιότητας ή/και της κατανομής των πιθανοτήτων των αβέβαιων στοιχείων του μοντέλου, ii) παραγωγή τιμών για τα αβέβαια στοιχεία του μοντέλου, iii) υπολογισμός των αποτελεσμάτων του μοντέλου και περιγραφή της κατανομής τους. Η ανάλυση ευαισθησίας περιλαμβάνει ένα επιπλέον βήμα (iv) που αφορά στον υπολογισμό των δεικτών ευαισθησίας. Στην περίπτωση που αρκετές εξισώσεις μοντέλων είναι διαθέσιμες για την πρόβλεψη μιας δεδομένης ποσότητας, ένα επιπλέον βήμα είναι να αναλυθεί με ειδικές τεχνικές η αβεβαιότητα που αφορά στις εξισώσεις του μοντέλου. Αρκετές από αυτές τις μεθόδους δοκιμάστηκαν χρησιμοποιώντας ως πρότυπο το μύκητα *Sclerotinia sclerotiorum*. Τα αποτελέσματα έδειξαν ότι μια μέτρια αβεβαιότητα στις τιμές των παραμέτρων μπορεί να προκαλέσει μια μεγάλη αβεβαιότητα στα αποτελέσματα του μοντέλου.

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Response of young olive trees to nitrogen fertilization

Y.E. Troyanos and E. Roukounaki

Summary The response of young olive trees to soil nitrate nitrogen imposed by different N fertilization rates and to a foliar N–P-K fertilizer was investigated in a pot experiment. The dry weight of leaves increased with increasing soil N fertilization rate, whereas that of the whole plants sprayed with a foliar N-P-K was not affected. The root length increased in N-deficient olives (e.g. olives grown without N fertilization) indicating that the N-deficient olives produced a longer root system. However, this longer root system was accompanied by a reduction in (stems+leaves+shoots) : root length ratio. When the leaf N concentration was <2% and the soil nitrate nitrogen <25 mg/kg DWT, the plants had the lowest leaf dry weight.

Additional Keywords: leaf dry weight, nitrate, *Olea europaea* L., root length

Introduction

In Greece, farmers are investing to modern olive growing by using drip-irrigation, fertigation and no-tillage cropping system for reducing soil erosion. However, fertilization of olive trees is based mostly on tradition, i.e. few growers are following soil and leaf analyses for the application of fertilizers, whereas most of them use only their empirical knowledge as a guide. During the establishment of young olive trees, fertilization practices applied by the growers are quite diverse. Some growers apply large quantities of nitrogen (N) fertilizers, while others none. The former may over-fertilize the olive trees causing potential growth reduction and toxicities. The latter claim that, without fertilization the young olive trees produce a large root system that penetrates deeper into the soil, which is desirable especially when there is water shortage (e.g. in rainfed conditions). However, in that case, olive trees are under-fertilized and growth reduction could be evident.

Response to N fertilization of young olive trees grown in soil (8) and nutrient solutions (13) has been reported in the literature. However, there is a discrepancy concerning the effect of N fertilization on mature olive trees. Hartmann (14) reported that mature olive trees responded to N only when grown in poor soil. Other researchers (5, 6) have shown that the traditional fertilization based on annual applications of N-P-K had no effect on yield of mature olive trees compared to the leaf analysis-based fertilization, which had a positive effect on yield. However, mature olive trees have been found to respond to foliar application of urea (2, 5, 17, 19) and potassium (18). In recent years, foliar feeding has been used extensively in olive orchards, especially under rain-fed conditions, where shortage of soil moisture reduces the availability of fertilizers (7).

In the present study, an experiment was carried out to investigate the response of young olive trees to N fertilization. Different rates of soil N fertilization and a foliar N-P-K fertilizer were applied to young olive trees (cv. 'Koroneiki'). The objectives were a) to determine the minimum soil NO_3-N concentration ([NO_3-N]) and the minimum leaf N concentration ([N]) for maximum growth, b) to determine the minimum N, P and K root absorption rates required for maximum growth of young olive trees, and c) to test if foliar feeding with a N-P-K fertilizer could sustain the maximum growth of plants. The

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effects of soil and foliar fertilization on olive tree growth and leaf [N] are discussed.

Material and Methods

Two hundred sixteen (216) one-year-old micro-propagated olive trees (cv. 'Koroneiki'), grown in plastic bags filled with a mixture of compost and perlite, were transplanted on 16 March 2007 into 14 l plastic pots to facilitate the access to their root system during their growth. The plastic pots contained a clay loam soil with the following characteristics: pH = 7.7, Electrical Conductivity (EC) = 0.66 dS/m (Saturated Paste), Organic matter = 1%, $CaCO_3$ = 29%, P = 3 ppm (Olsen method), K = 156 ppm (extraction with 1 M NH_4OAc at pH = 7.0) and B = 1.3 ppm (extraction with hot water). Prior to transplanting, the length of the new shoots and the length and diameter of stems were measured. These measurements were used to estimate the initial size of the plants.

Following their transplanting, the experimental olive trees were placed outdoors with the pots covered with plastic bags to reduce water evaporation and to ensure that no rain water would enter the pots. The young olive trees were irrigated using tap water (NO_3-N concentration = 1.1 ppm, EC = 0.915 dS/m) and received no fertilization for one month following their transplanting. The soil moisture was monitored using tensiometers and water was applied to the soil until field capacity. The mean air temperatures during the experiment are given in Figure 1.

According to their size, the experimental young olive trees were sorted in descending order. The trees were then split into 6 groups, with the 1st group consisting of the largest ones and the 6th group of the smallest. The 36 plants in each group were then randomly assigned to 6 rows and columns according to a 6x6 Latin Square design. This randomization reduced the effect of variation in the initial size of the plants on their final growth (1). Five different soil N fertilization rates, 0 (N_0), 0.95 (N_1), 1.90 (N_2), 3.80 (N_3) and 6.25 (N_4) g N (Table 1) and a foliar treatment of 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer (N_f), as recommended by the manufacturer, were gradually applied to each plant. During the course of the experiment, a total of six destructive harvests were carried out 31, 65, 93, 136, 167 and 201 days after the first fertilization (DAF) by randomly selecting 36 plants (6 treatments x 6 replications) at each harvest.

Based on the results of soil analyses, on 15 April 2007, 0.74 g KH_2PO_4 and 1.33 g K_2SO_4 dissolved in 1 l tap water were applied to each pot of the treatments N_0, N_1, N_2, N_3

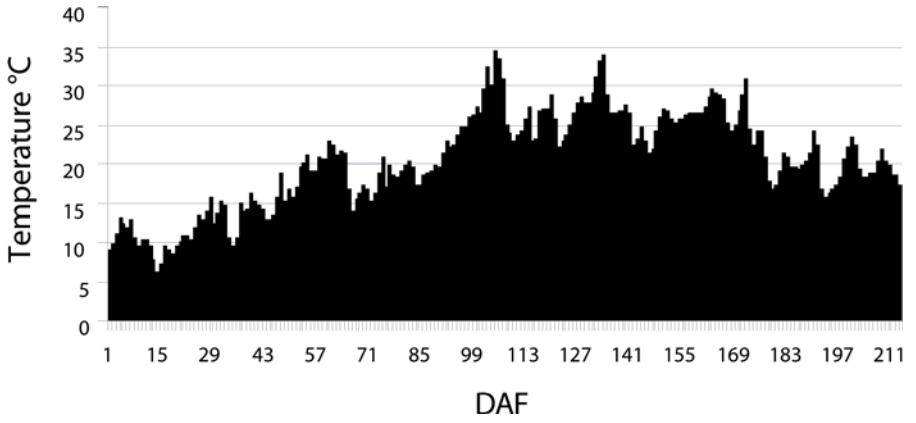

Figure 1. Changes in the mean daily temperature (°C) during the course of the experiment. DAF: days after first fertilization.

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Table 1. Soil N fertilization rates applied monthly to one-year-old olive trees.

Treatments	Soil N fertilization rates (g/pot)			
	16 April 2007	15 May 2007	15 June 2007	Total
N_0	0	0	0	0
N_1	0.45	0.50	0	0.95
N_2	0.45	0.45	1	1.90
N_3	0.45	1.35	2	3.80
N_4	0.45	1.80	4	6.25

and N_4, whereas in pots of treatment N_f no soil fertilizer was applied. The following day (16 April 2007), 0.45 g N, derived from NH_4NO_3 and dissolved in 1 l tap water, were applied to each pot of the treatments N_1, N_2, N_3 and N_4, whereas no N was applied to pots of treatment N_0 (Control). On the same day, the olive trees of treatment N_f were sprayed with 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer. The rest of the soil N fertilizer was gradually applied according to Table 1, whereas the olive trees of treatment N_f received a total of six monthly foliar applications.

Statistical analyses

The statistical analyses were carried out using Genstat (10th Edition). ANOVA was applied and when the F-test was statistically significant ($P<0.05$) the comparisons of means were performed using the Duncan and the LSD (Least significant differences) tests. Non-linear regression analysis was performed between the leaf dry weight (DWT) and the soil [NO_3-N].

Harvests - Measurements

One week prior to each harvest, 36 soil samples (6 treatments x 6 replications) were taken from the pots using a soil auger. The samples were stored in a refrigerator and the following day the soil NO_3-N was extracted using a 1:10 w/w soil to deionized water ratio. NO_3-N was determined using a modified hydrazine nitrate reduction method (21). Each soil sample was extracted and analysed twice and if the results differ more than 10%, a third sub-sample was analysed and the mean of the three measurements was estimated. The dry weight (DWT) of the soil samples was determined after drying at 105°C until constant weight and the soil [NO_3-N] was expressed on a dry weight basis.

At each harvest, the above ground plant parts, i.e. leaves, stems and shoots, were cut, placed separately into plastic bags to reduce moisture loss and weighted (fresh weight). The length of the shoots and the diameter of the stems were also measured. The following day, the roots were removed from each pot and washed from the soil using a sieve mesh; then they were placed into plastic bags and put in a refrigerator until the root length was measured. The root length was measured according to the method of Tennant (20) using square grids of 1 cm (for the first harvest) and 2 cm (for the rest 5 harvests). All plant parts were washed using deionised water and dried by placing them into an air-forced oven at 80°C until constant weight.

After drying, the plant samples were ground and 100 mg of each sample were digested with 2 ml H_2SO_4 containing 1 g/l Se and 1 ml 30% H_2O_2. The digests were made up to 25 ml with deionized water and the concentration of ammonium-N was determined using the indophenol blue method (22). There were two digestions per treatment and replication and if the results differ more than 10%, a third sub-sample was analysed and the mean of the three measurements was taken.

Results and Discussion

At 201 DAF (last harvest), the DWT of leaves responded to N fertilization treatments (Fig.

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2), whereas the DWT of shoots, stems and roots did not (data not shown). The leaf DWT of the plants grown with treatment N_f was less than that of the plants grown with N_1, N_2, N_3 and N_4, treatments whereas that of young olive trees grown with no N fertilization (N_0) had a intermediate value. The response of olive cv. 'Koroneiki' to increased soil N fertilization rate has been reported to be different than that of other cultivars, such as Nabali, Manzanillo, etc. (8). More specifically, in the latter cultivars, the increased N availability increased the shoot DWT and length, but it did not have any effect on the leaf DWT (8). These results show that varietal differences to N fertilization response could be expected.

In the present study, the root length was the greatest in the non-fertilized (N_0) olive trees and it was reduced with increasing soil N fertilization rate (Fig. 3). The root length of young olives treated with leaf application of N (treatment N_f) did not differ significantly ($P<0.05$) from that of the trees treated with N_3 and N_4. The increased root length with diminishing soil N external supply has been recorded in other plants too and it is probably one of the mechanisms by which plants adjust to shortage of exogenous resources (15). The results of the present study are in agreement with field empirical observations made by growers according to which, olive trees grown without nitrogen fertilizer produced longer roots. However, in the current experiment the increased root length, observed in the N-deficient young olive trees (Fig. 3), was accompanied by a reduction in the leaf+stems+shoots DWT: root length ratio (Fig. 4) which is undesirable, especially during the establishment of young olive trees.

The leaf [N] increased ($P<0.01$) with increasing N soil supply (Fig. 5). Leaf [N] was relatively stable during time in treatments N_2, N_3 and N_4, whereas in treatments N_0 and N_f it decreased considerably from 65 DAF showing that soil and foliar fertilization did not satisfy the N requirements of young olive trees. Response of leaf [N] of young olive trees to N fertilization has been reported elsewhere (3, 8, 13, 19). At 201 DAF, when the leaf DWT of the experimental plants grown without fertilization (N_0) was affected by the soil N fertilizer treatments, the leaf [N] was approximately <2%, indicating that this leaf [N] was not sufficient for the growth of the young olive trees. Nevertheless, this concentration has been reported to be sufficient for

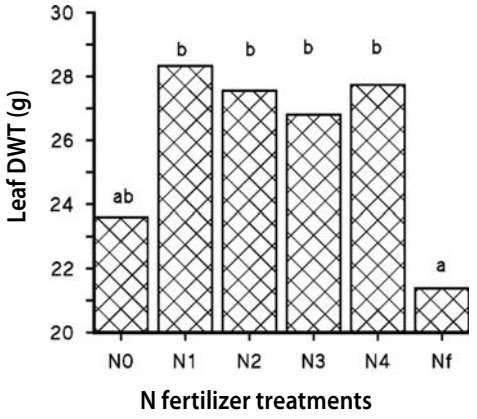

Figure 2. Effects of N fertilization on olive leaf dry weight (DWT), 201 days after first fertilization (DAF). Soil N fertilization rates: N_0: 0, N_1: 0.95, N_2: 1.90, N_3: 3.80, N_4: 6.25 g N at three monthly applications. Foliar treatment N_f: 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer at six monthly applications. Means followed by a different letter are significantly different at $P<0.05$ level. Means separation calculated by the Duncan test.

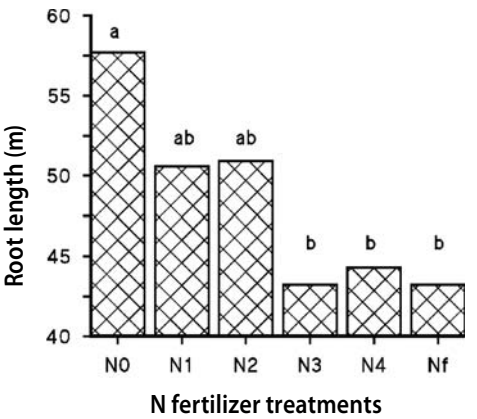

Figure 3. Effects of N fertilization on root length (m) of one-year-old olive trees. Soil N fertilization rates: N_0: 0, N_1: 0.95, N_2: 1.90, N_3: 3.80, N_4: 6.25 g N at three monthly applications. Foliar treatment N_f: 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer at six monthly applications. Means followed by a different letter are significantly different at $P<0.05$ level. Means separation calculated by the Duncan test.

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the growth of old mature olive trees (4, 13). However, it has also been reported that the optimum leaf [N] is higher in young than in mature olive trees (16). Furthermore, Gonzalez *et al*. (10, 11, 12) found that the yield of mature olive trees was reduced when leaf [N] was < 1.95%.

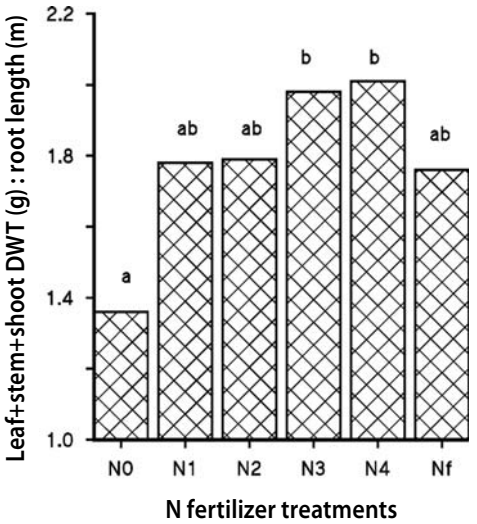

Figure 4. Effects of N fertilization on the ratio of leaves+shoots+stems dry weight (DWT) (g) : root length (m) of one-year-old olive trees. Soil N fertilization rates: N_0: 0, N_1: 0.95, N_2: 1.90, N_3: 3.80, N_4: 6.25 g N at three monthly applications. Foliar treatment N_f: 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer at six monthly applications. Means followed by a different letter are significantly different at P<0.05 level. Means separation calculated by the Duncan test.

The results of the present study also showed that the increased leaf DWT was accompanied by a significant (P<0.01) increased soil [NO_3-N] due to split soil N fertilization, nitrification and addition of NO_3-N with irrigation (Fig. 6). After the last application of soil N fertilizer (65 DAF), big differences (P<0.05) in soil [NO_3-N] were found among the treatments. The soil [NO_3-N] in treatments N_3 and N_4 was very high (>100 mg/kg DWT), whereas in the other treatments it was between 20 and 50 mg/kg DWT. To estimate the threshold soil [NO_3-N] (25 mg/kg DWT) for maximum growth of young olive trees, the relationship between leaf DWT and soil [NO_3-N] was used (Fig. 7). The relationship between the mean leaf DWT and the mean soil [NO_3-N] during the course of the experiment was curvilinear and it was described by the following equation:

$$\text{Leaf DWT} = a+b*r^{[NO_3\text{-N}]}+c^{[NO_3\text{-N}]}$$

where, a, b, r and c are the coefficients derived from the statistical analysis (Table 2).

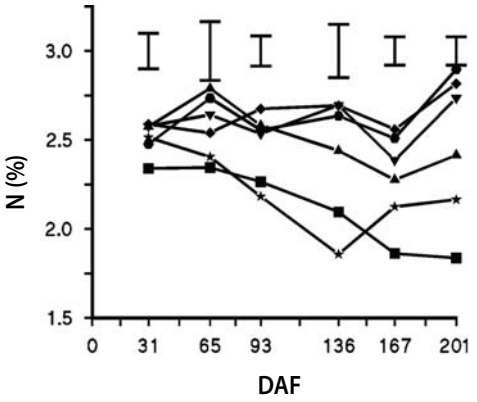

Figure 5. Changes in the leaf [N] (%) during the growth of one-year-old olive trees. DAF: days after first fertilization. Soil N fertilization rates: N_0: 0 (■-■), N_1: 0.95 (▲-▲), N_2: 1.90 (▼-▼), N_3: 3.80 (♦-♦), N_4: 6.25 (●-●) g N at three monthly applications. Foliar treatment N_f: 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer at six monthly applications. Bars: Least significant difference (LSD) at each harvest.

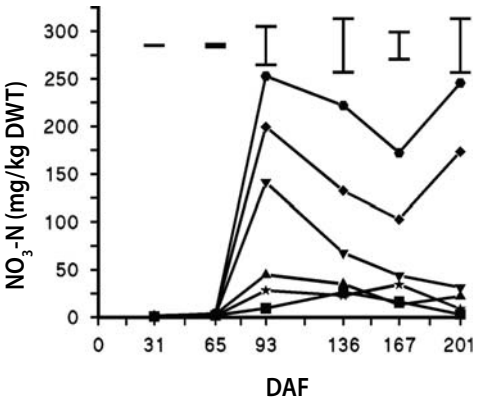

Figure 6. Changes in the soil [NO_3-N] (mg kg^{-1} DWT) during the growth of one-year-old olive trees. DAF: days after first fertilization. Soil N fertilization rates: N_0: 0 (■-■), N_1: 0.95 (▲-▲), N_2: 1.90 (▼-▼), N_3: 3.80 (♦-♦), N_4: 6.25 (●-●) g N at three monthly applications. Foliar treatment N_f (*-*): 0.3 g/l of 21-21-21 (N-P-K) soluble fertilizer at six monthly applications. Bars: Least significant difference (LSD) at each harvest.

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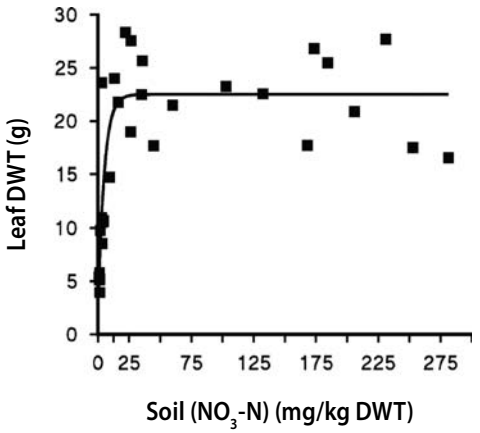

Figure 7. Relationship between the mean leaf dry weight (DWT) (g) of one-year olive trees and the mean soil concentration of NO_3-N (mg/kg DWT) during the course of the experiment.

Table 2. Relationship between the mean leaf dry weight (DWT) (g) and the mean soil concentration of NO_3-N (mg/kg DWT) during the course of the experiment.

Leaf DWT = $a+b*r^{[NO_3\text{-}N]}+c^{[NO_3\text{-}N]}$		
Estimated coefficients		SE
a	0.821	0.071
b	-22.180	3.76
r	-0.008	0.013
c	23.410	1.85
R = 71.7 %, P<0.001		

The young olive trees responded to N when the N fertilization rate was >0.95 g N/pot and soil [NO_3-N] was ≤25 mg/kg DWT. The young olive trees could resist high soil [NO_3-N] without growth reduction. For example, there was no reduction in leaf growth of the experimental trees when the soil N fertilization rate was increased from 1.90 (N_2) to 6.25 (N_4) g/pot (Fig. 2) causing an increase in soil [NO_3-N] from 50 to 300 mg/kg DWT (Fig. 6). However, in high soil N fertilization rates, i.e. N_3 and N_4, the leaf+stem+shoot DWT: root length ratio was low indicating that the plants had a shorter root length, which is undesirable for olive trees grown under rain-fed conditions. The foliar application of N-P-K produced the lowest olive leaf DWT (Fig. 2). However, the reduced growth could not be attributed only to N but also to P and K, since in the present study, none of these two nutrients was applied to soil.

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Αντίδραση νεαρών δενδρυλλίων ελιάς στην αζωτούχο λίπανση

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Περίληψη Η αντίδραση νεαρών φυτών ελιάς (ποικ. 'Κορωνέϊκη) στην αυξανόμενη συγκέντρωση του νιτρικού αζώτου, μετά από εφαρμογή διαφορετικών ποσοτήτων αζωτούχου λιπάσματος στο έδαφος και ενός λιπάσματος N-P-K στα φύλλα, μελετήθηκε σε ένα πείραμα που πραγματοποιήθηκε σε γλάστρες. Τα αποτελέσματα έδειξαν ότι το βάρος της ξηράς ουσίας των φύλλων αυξήθηκε με την αύξηση της ποσότητας της αζωτούχου λίπανσης στο έδαφος, ενώ το βάρος της ξηράς ουσίας των φύλλων δεν επηρεάστηκε στα φυτά που δέχτηκαν το διαφυλλικό N-P-K λίπασμα. Επιπλέον, στα φυτά που αναπτύχθηκαν σε συνθήκες έλλειψης αζώτου (δηλ. φυτά στα οποία δεν έγινε εφαρμογή αζωτούχου λίπανσης στο έδαφος), το μήκος της ρίζας αυξήθηκε γεγονός το οποίο οδηγεί στο συμπέρασμα ότι η έλλειψη αζώτου σε νεαρά φυτά ελιάς προκαλεί την παραγωγή μεγαλύτερου μήκους ρίζας. Η αύξηση όμως στο μήκος της ρίζας συνοδεύτηκε από ανεπιθύμητη μείωση της αναλογίας του ξηρού βάρους του υπέργειου τμήματος των φυτών (νεαρών βλαστών + κορμού + φύλλων) : μήκος της ρίζας. Τα αποτελέσματα της παρούσας μελέτης έδειξαν επίσης ότι, όταν η συγκέντρωση του N στην ξηρά ουσία των φύλλων ήταν <2% και η συγκέντρωση του νιτρικού αζώτου στο ξηρό βάρος του εδάφους ήταν <25 mg/kg, τα νεαρά δενδρύλλια ελιάς είχαν την μικρότερη ανάπτυξη.

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SHORT COMMUNICATION

The effect of a garlic essential oil component and entomopathogenic nematodes on the suppression of *Meloidogyne javanica* on tomato

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Summary Root-knot nematodes are worldwide distributed plant pests with a wide range of hosts that cause downgrading and unmarketability of produce, significant yield decrease, or even total failure of various crops. The entomopathogenic nematodes have extensively been studied as a potential alternative method for the control of plant parasitic nematodes. In addition, the essential oil of garlic and its volatile components that possess fumigant properties against several plant pests and pathogens have also been shown to suppress plant parasitic nematodes. The present work is a pilot study examining the possibility of a combined action of *Steinernema carpocapsae* and diallyl disulfide, a volatile component of garlic essential oil, against *Meloidogyne javanica*. The results of the study showed that the combined use of *S. carpocapsae* and diallyl disulfide significantly reduced the population of *M. javanica* on tomato.

Additional keywords: diallyl disulfide, root-knot nematodes, *Steinernema carpocapsae*

Root-knot nematodes (*Meloidogyne* Goldi 1892 - RKN) are obligate parasites of higher plants distributed worldwide causing considerable yield losses and reduction of product quality on almost every plant species. Garlic essential oil and its volatile components have repeatedly been studied and it is now commonly accepted that they possess fumigant properties against several plant pests and pathogens, including plant parasitic nematodes (PPN) (5, 7). Diallyl disulfide, used in the present study, is one of the garlic essential oil volatile components that accounts for 30-50% of the total sulphide mixture (13). Entomopathogenic nematodes (EPN) (*Heterorhabditis* and *Steinernema* species) are obligate parasites of insects that kill their hosts by introducing their bacterial symbionts (*Photorhabdus* and *Xenorhabdus* species, respectively) into the insect's haemocoel (2, 4). Surprisingly, some 25 years ago, it was shown that the co-existence of PPN and EPN causes a reduction in PPN populations (3, 6, 10). The objective of the present study was to examine whether there is a possibility of a combined action of EPN and diallyl disulfide in suppressing RKN.

The *Meloidogyne javanica* (*Mj*) inoculum was produced on tomato plants cv. 'Belladona', maintained in a growth chamber at 25°C for two months. Egg masses of *Mj* were randomly hand-picked from the infected tomato roots and used immediately for soil inoculation.

Steinernema carpocapsae (Sc) (Koppert B.V. Berkel eb Rodenrijs, The Netherlands®) was reared on *Galleria melonella* (Lepidoptera: Pyralidae) at 25°C (8). Infective juveniles were recovered using White traps

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(14) and stored in 1 l flasks filled with tap water at 4°C (approximately for 5-7 days) until further use. For the trials with live infective juveniles (IJ) and prior to the soil inoculation, the nematodes were left for 30-60 min at room temperature in order to recover. For the trials with dead IJ, the nematodes were heat-killed prior to soil inoculation on a heat block at 70°C for 15 min.

Diallyl disulfide (purity 70%) was purchased from Across Organics (New Jersey, USA). Laboratory-based gas chromatography analysis revealed two other main components: diallyl sulfide (15%) and diallyl trisulfide (12%).

Tomato seedlings (cv. 'Belladona') with three pairs of leaves were transplanted in 250 cm^3 plastic transparent pots containing commercial compost soil. After two days, groups of five egg masses (about 2,000 eggs, as estimated after dissolving the egg masses with sodium chloride) were added to the pots. All EPN treatments were added simultaneously with *Mj* at a rate of 7,000 live *S. carpocapsae* (Sc) or 7,000 heat-killed. Each pot received 20 ml of 2 µl/ml diallyl disulfide solution (Dd) in a single or a double application, i.e. concurrent with *Mj* inoculation (single application) or concurrent with and one week after *Mj* inoculation (double application). Control pots received 20 ml of distilled water.

The experiment consisted of eight treatments (plus control) and each treatment was replicated five times in a completely randomized experimental design.

Experimental plants were incubated in a growth chamber at 25°C. After 28 days, the roots were submerged in water to gently rinse away the soil. The roots were dried off on tissue paper and their fresh weight was measured. Subsequently, the roots were cut into 1-2 cm pieces and females of *Mj* were teased from the roots and counted at 17.5x magnification. Statistical analysis was performed with SAS software (SAS Institute, Cary, NC) and mean separation was conducted using the Tukey's test (12).

The number of female *Mj* counted in roots treated with live or dead Sc and/or Dd was significantly lower than that in the untreated control ($P<0.001$). The greatest disparity was observed when Dd was applied in a double application (at 0 and 7 days post-*Mj* inoculation) to soil previously treated with dead or live Sc. No statistically significant ($P<0.001$) differences were noted between the treatments with live and dead Sc. However, in both treatments, the number of *Mj* females was reduced by 32% and 45%, respectively, compared to the control. The total numbers of *Mj* females counted in tomato roots treated with Dd in a single or a double application, were significantly ($P<0.001$) lower than those in the control. In treatments with dead Sc + Dd, the number of *Mj* females that developed from egg masses in the Dd double application was significantly ($P<0.001$) lower than that in the single application. In treatments with live Sc + Dd, the number of *Mj* females developed from egg masses in the Dd double application was not significantly ($P<0.001$) different to that of the single application (Table 1). No statistically significant differences were noted with regard to root weight ($P>0.05$). The results of the present study showed that both live and dead IJ of Sc suppressed *Mj* on tomato plants, which is in accordance with the findings of Lewis *et al.* (9). In contrast to these results, Grewal *et al.* (6) found no effect of live IJ, which may indicate that these nematodes act by a slower release of the intestinal bacterial agents induced by their natural death. Bird and Bird (3) also suggested that PPN suppression may be due to a competition for habitat and space. These factors may influence the effectiveness of live IJ, depending on the environmental conditions, the plant parasitic and entomopathogenic nematode species, the soil type and the host plant or the presence/absence of insect hosts. The results of the present study are in agreement with previous research on nematode suppression by garlic seeds and bulbs, garlic essential oil and garlic essential oil components (1, 6). Diallyl disulfide was more effective when it was used in two applications, one concurrent with *Mj* inoculation and a second one 7 days later. It is likely that some eggs that

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Table 1. Effects of diallyl disulfide (Dd), used alone or in combination with dead or live *Steinernema carpocapsae* (Sc), on the number of female *Meloidogyne javanica* (*Mj*), 28 days after inoculation of potted tomato plants (cv. 'Belladona').

Treatment	Rate/dose	Time of application (dpi)[1]	Nematodes per root [2]
Control	0	0	243 a[3]
Dd	2 µl/ml	0	112.8 cd
Dd	2 & 2 µl/ml	0 & 7	76.4 def
Live Sc	7,000 IJ	0	165.4 b
Dead Sc	7,000 IJ	0	133 bc
Live Sc + Dd	7,000 IJ + 2 µl/ml	0	104.2 cde
Live Sc + Dd	7,000 IJ + (2 & 2 µl/ml)	0 + (0 & 7)	71.2 ef
Dead Sc + Dd	7,000 IJ + 2 µl/ml	0	101 cde
Dead Sc + Dd	7,000 IJ + (2 & 2 µl/ml)	0 + (0 & 7)	59.4 f
LSD = 40			

[1]dpi: days post-*Mj* inoculation
[2]Mean of five replicates
[3]Numbers followed by the same letter do not differ significantly at P<0.001

survive the first application hatch into infective juveniles, which are subsequently killed by the second application. Alternatively, due to the high volatility of the diallyl disulfide and the protective nature of the egg mass (11), repeated applications are required to achieve higher effectiveness. It is also worth mentioning that neither the single nor the double application of diallyl disulfide caused any phytotoxicity.

It can be concluded that both *S. carpocapsae* and diallyl disulfide exhibit significant nematicidal or nematostatic properties and have the potential for nematode control. However, more parameters should be studied, such as plant parasitic nematode initial infestation density, soil type, application time, dosage and repeated applications of diallyl disulfide.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Επίδραση ενός αιθερίου ελαίου του σκόρδου και εντομοπαθογόνων νηματωδών στην καταστολή του *Meloidogyne javanica* στην τομάτα

Ι. Αναστασιάδης, Α.Κ. Κυμπάρης, Μ. Κορμπή, Μ.Γ. Πολυσίου και Ε. Καραναστάση

Περίληψη Οι νηματώδεις του γένους *Meloidogyne* έχουν παγκόσμια εξάπλωση και πολύ μεγάλο εύρος ξενιστών, στους οποίους μπορεί να προκαλέσουν υποβάθμιση της εμπορευσιμότητας του παραγόμενου προϊόντος, σημαντική μείωση της παραγωγής, και σε πολλές περιπτώσεις ακόμη και την ολική καταστροφή της καλλιέργειας. Οι εντομοπαθογόνοι νηματώδεις είναι οργανισμοί που έχουν μελετηθεί εκτενώς ως προς τη δυνατότητα χρησιμοποίησής τους ως εναλλακτικών μεθόδων αντιμετώπισης των φυτοπαρασιτικών νηματωδών. Επίσης, το αιθέριο έλαιο του σκόρδου, το οποίο διαθέτει απολυμαντικές ιδιότητες εναντίον πολυάριθμων εχθρών και ασθενειών των φυτών, έχει ήδη αποδειχτεί ότι μπορεί να καταστέλλει και τη δράση των νηματωδών. Η παρούσα εργασία αποτελεί μια πιλοτική μελέτη που σκοπό έχει να διερευνήσει την πιθανότητα συνδυαστικής δράσης του εντομοπαθογόνου νηματώδη *Steinernema carpocapsae* και του διάλλυλο δισουλφιδίου, ενός πτητικού συστατικού του αιθερίου ελαίου του σκόρδου, για την αντιμετώπιση του *Meloidogyne javanica*.

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SHORT COMMUNICATION

Root-knot nematodes (*Meloidogyne* spp.) in Greece

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Summary The information presented in the current work on the occurrence of root-knot nematodes (*Meloidogyne* spp.) in Greece was extracted from the literature and unpublished studies conducted by the authors. The species *M. javanica, M. incognita*, *M. arenaria*, *M. hapla*, *M. artiellia* and *M. exigua* had been reported during the period 1963-1994 to occur on various host plants and species identification was based on morphological characters. Since 1996, 52 isolates from Crete and 9 isolates from the mainland of Greece were identified using molecular and/or biochemical markers. The species found were *M. javanica, M. incognita* and *M. arenaria*. Twenty-six of these isolates were identified as *M. javanica* (19 isolates), *M. incognita* (5 isolates) and *M. arenaria* (2 isolates) on the basis of the esterase phenotypes. All *M. javanica* isolates exhibited the typical J3 phenotype except one from Crete, which exhibited the J2 phenotype. The *M. incognita* and *M. arenaria* isolates revealed the I1 and A2 phenotypes, respectively. Finally, the infestation of potato tubers by a *M. javanica* isolate (phenotype J3) is reported for the first time in Greece.

Additional key words: esterase phenotypes, *Meloidogyne arenaria, M. incognita, M. javanica*, potato

Phytonematology is a relatively new science in the Mediterranean region. It was first developed during the 1950s as an experimental discipline in some countries and had an increasing impetus in the following years (27). Root-knot nematodes (RKN), *Meloidogyne* spp., are amongst the most economically important nematodes in agriculture, exhibiting a broad host range (6) and a wide distribution in the Mediterranean basin (27). In Greece, RKN have been recorded in several areas and till the mid 90's, species identification had been based on morphological and morphometric characters and/or differential host tests. The objective of the present work was to report on the status of the occurrence of RKN in Greece. The Helminthological Abstracts, Series B, Plant Nematology (Nematological Abstracts from 1992) and other publications accessible/available to the authors were the sources of information used in the current work, along with some, so far, unpublished studies conducted by the authors. Data of the *Meloidogyne* species identified during the period 1963-1994 and the hosts on which they had been detected are presented in Table 1. Published reports, in which the specific host-nematode associations were not clearly determined and information extracted from abstracts, when the full text of the respective papers was not available to the authors, are also presented below.

The review of the status of *Meloidogyne* spp. in Greece until 1979 includes species associated with at least 85 host plants and listed in Table 1 (7, 8). As *M. thamesi* is synonymous to *M. arenaria* (2), these two species are listed together. In Crete, *M. javanica*, *M. incognita* and *M. arenaria* were detected throughout the coastal region-below 200 m altitude-

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whereas, *M. hapla* was found only in one location above 450 m altitude (28, 29). The last report of the occurrence of *Meloidogyne* spp. in Greece was in 1994 and referred to the presence of *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* on anemone, apricot, carnation, celery, courgette, cucumber, aubergine, kiwi, peach, potato, red pepper, rose and tomato, without any further details on specific host associations (36).

During the period 1996-2010, 61 isolates of *Meloidogyne* spp. were sent to the Plant Protection Institute of Heraklion, N.AG. RE.F. (Crete, Greece). Fifty-two of those isolates were collected from greenhouses and fields in Crete and 9 isolates from the mainland of Greece (Table 2). The isolates were identified at the Scottish Crop Research Institute (Dundee, UK) and/or the Instituto do Ambiente e Vida, Departamento de Zoologia, Universidade de Coimbra (Portugal), using RAPD, IGS-PCR, SCAR-PCR and esterase phenotypes. Some of those isolates were also characterised by their perineal pattern morphology at both the Instituto do Ambiente e Vida (Portugal) and the Plant Protection Institute of Heraklion (Greece). The esterase phenotypes were used to identify 26 *Meloidogyne* isolates (19 of *M. javanica*, 5 of *M. incognita* and 2 of *M. arenaria*). All *M. javanica* isolates had the typical J3 phenotype except one from Crete, which had the J2 phenotype. *M. incognita* exhibited the I1 phenotype and the two *M. arenaria* isolates, collected from balm (*Melissa officinalis* L.) in Thrace and grapevine (*Vitis vinifera* L.) in Crete, respectively, had the A2 phenotype, one of the three characteristic phenotypes of the species (5). As in the present work, no specimens from wheat or peach were collected, two *Meloidogyne* species, namely *M. artiellia* and *M. exigua,* reported in the past to occur in Greece (19, 9), were not found. *M. hapla* was not found either, although during

Table 1. Root-knot nematodes (*Meloidogyne* spp.) and their associated hosts reported in Greece during the period 1963-1994.

Meloidogyne species	Hosts (in alphabetical order)	References*
M. arenaria (syn. *M. thamesi*)	Antirrhinum, aubergine, bean, brindweed, cabbage, carrot, corn, cucumber, garlic, geranium, grapevine, hyacinth, leek, lettuce, melon, okra, parsley, peanut, pelargonium, poppy, potato, snapdragon, tobacco tomato, trout lily, zerbera	1, 4, 9, 11-14, 16, 18, 26**
M. artiellia	Wheat	19
M. exigua	Peach	9
M. hapla	Bean, cyclamen, kiwi, leek, tomato	1, 26, 34, 35, 37
M. incognita (syn. *M. incognita acrita*)	Almond, aubergine bean, carrot, cotton, cucumber, fig, fuchsia, gardenia, grapevine, hyacinth, okra, peach, pepper, potato, rose, sugarbeet, tobacco, tomato, watermelon	1, 3, 4, 10, 12-18, 20-25
M. javanica	Almond, aubergine, banana, bean, beets, black salsify, carrot, celery, cyclamen, fig, hyacinth, kiwi, okra, olive, peach, pepper, pistachio, plum, pomegranate, sugarbeet, tobacco, tomato, grapevine	1, 3, 4, 9-11, 16, 17, 23, 25, 30, 34, 35, 38

*Additional information can be found in reference 36, which lists also hosts of *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* without further details on species associations.
**Reference 26 cites data from 1964.

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Table 2. Root-knot nematodes (*Meloidogyne* spp.) and their associated hosts reported in Greece during the period 1996-2010.

Meloidogyne species	Hosts (in alphabetical order)	Locality*	References
M. arenaria	Balm, grapevine	Crete (1) Thrace (1)	5
M. incognita	Cucumber, tomato, pepper	Crete (8) Peloponissos (1)	31-33 and unpublished data
M. javanica	Aubergine, balm, banana, bean, cabbage, carnation, cucumber, grapevine, melon, ornamentals, potato (tubers), tomato	Crete (43) Epirus (4) Thessaly (1) Thrace (1) Peloponissos (1)	31-33 and unpublished data

* Figures in parentheses refer to the number of isolates

the period 1966-1994, there had been numerous reports of its occurrence in Greece. It is likely that *M. hapla* has a more restricted distribution in Greece compared to the other three major *Meloidogyne* species and is probably rare in Crete from where most of the isolates (85%) originated.

Potato tubers infested with RKN have been observed in Greece twice in the past, but the *Meloidogyne* species involved were not identified. However, during the current work, one isolate, collected in March 2010 from infested potato tubers in a field in Southern Crete (Fig. 1) was multiplied on susceptible tomato plants and identified as *M. javanica* (J3), using the esterase phenotypes.

In addition to the list of RKN and their associated hosts recorded in Greece since 1963, the present work reports for the first time in Greece on (a) the infestation of potato tubers by *M. javanica* (phenotype J3), and (b) the presence of the two esterase phenotypes (J2 and J3) associated with *M. javanica* isolates.

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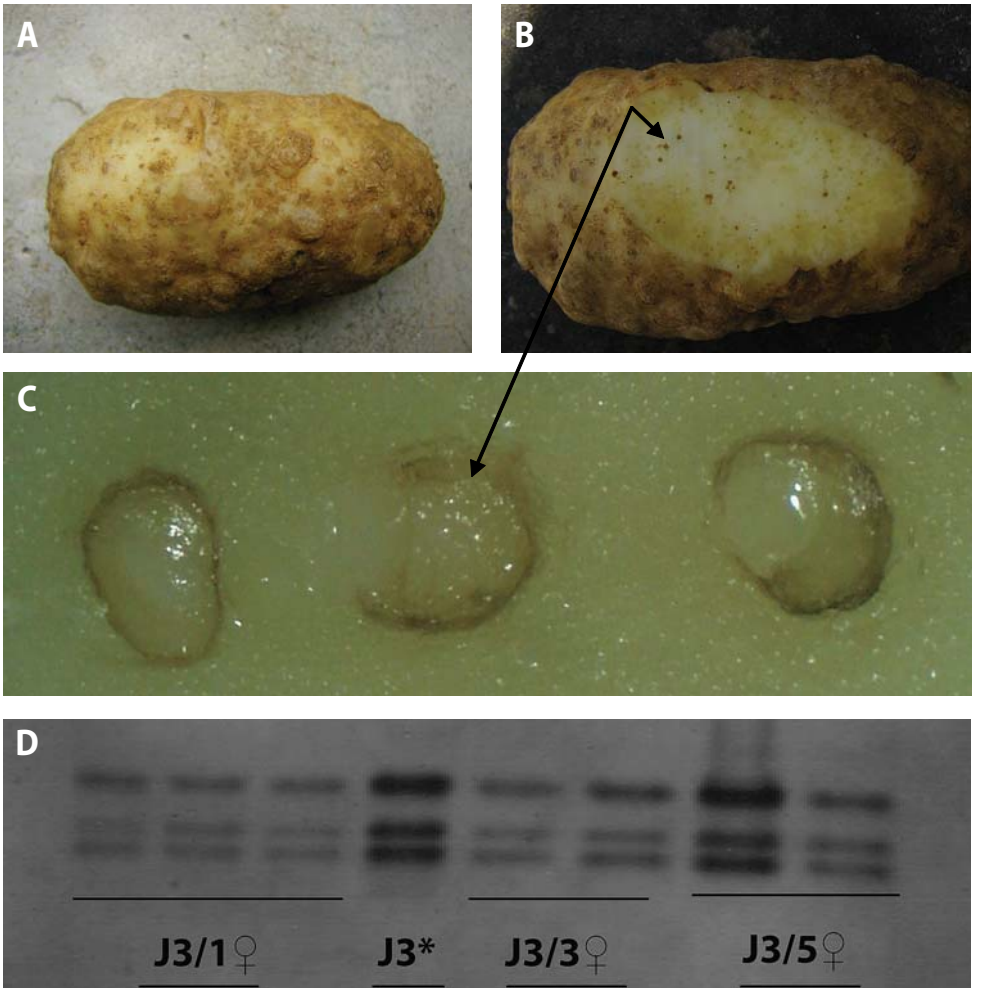

Figure 1. Potato tuber infested with *Meloidogyne javanica*. A: deformation of the outermost tuber layer. B & C: a section showing females and egg masses. D: esterase phenotypes of the *M. javanica* isolate detected in potato tubers from Crete (J3). *Reference population of *M. javanica* (J3).

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Νηματώδεις του γένους *Meloidogyne* στην Ελλάδα

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Περίληψη Στην παρούσα εργασία παρουσιάζονται συνολικά όλες οι καταγραφές νηματωδών του γένους *Meloidogyne* στην Ελλάδα κατά την περίοδο 1963-2010 με βάση την υπάρχουσα βιβλιογραφία και αδημοσίευτα στοιχεία από μελέτες των συγγραφέων. Τα είδη *M. javanica, M. incognita*, *M. arenaria*, *M. hapla*, *M. artiellia* και *M. exigua* αναφέρθηκαν σε διάφορους ξενιστές από το 1963 έως το 1994 και προσδιορίσθηκαν με βάση τα μορφολογικά χαρακτηριστικά τους. Από το 1996 μέχρι σήμερα ταυτοποιήθη-

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καν με μοριακές ή/και βιοχημικές μεθόδους 52 πληθυσμοί *Meloidogyne* spp. από την Κρήτη και 9 από την ηπειρωτική Ελλάδα. Τα είδη που βρέθηκαν ήταν τα *M. javanica, M. incognita* και *M. arenaria*. Σε 26 από τους παραπάνω πληθυσμούς προσδιορίστηκαν οι φαινότυποι της εστεράσης και οι πληθυσμοί χαρακτηρίστηκαν ως *M. javanica* (19 πληθυσμοί), *M. incognita* (5 πληθυσμοί) και *M. arenaria* (2 πληθυσμοί). Οι πληθυσμοί του είδους *M. javanica* είχαν τον τυπικό φαινότυπο J3 εκτός από έναν που προερχόταν από την Κρήτη και είχε τον φαινότυπο J2. Οι πληθυσμοί *M. incognita* και *M. arenaria* είχαν τους τυπικούς φαινοτύπους I1 και A2, αντίστοιχα. Η παρούσα εργασία καταγράφει για πρώτη φορά στην Ελλάδα προσβολή κονδύλων πατάτας από το είδος *M. javanica* (φαινότυπο J3).

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