Since its first cultivation and improvement at the beginning of the 19th century, sugarbeet (Beta vulgaris vulgaris L.) has gradually achieved prominence as a crop of economic importance both worldwide and at national level. Presently, it is potentially one of the most important crops in terms of economical viability and agricultural vitality for many countries. In Portugal, it is an alternative to traditional crops, with rising importance from both agronomic and economic points of view and following the rules of the new European common agriculture policy (M. Espadinha, DAI, personal communication).

Nematodes are among the most important pests that affect this crop, being responsible for 10% of the losses in the total amount of sugar produced (Steele, 1984; Sasser, 1989). Several species of nematodes feed on the roots or occasionally in the shoot tissues; young seedlings are very susceptible and may suffer irreversible damage (Cooke, 1993). Heterodera and Meloidogyne are the main genera responsible for the loss of productivity in sugarbeet fields (Steele, 1984; Shurtleff and Averre, 2000; Starr et al., 2002).

In general terms, research regarding relationships between phytoparasitic nematodes and soil variables from cultivated fields pays particular attention to the gender or species that is responsible for the greater amount of damage. However, and although the host plays a predominant role in the distribution and presence/absence of the individuals of this group, agricultural practices result in alterations of the physico-chemical soil parameters. These alterations condition the composition and distribution of the nematode community associated with the crop under consideration (Yeates, 1999).

To study the changes in population densities of phytoparasitic nematodes associated with sugarbeet cultivation and their relationships with various soil factors, a survey was conducted during the three final months of the fallow period after sugarbeet cultivation (February to April) and the first three months of the tomato cropping period (May to July), in southern Portugal.

**MATERIALS AND METHODS**

**Study area.** The study area is located in Portugal’s southern region, with the region’s typically temperate climate. The average air temperature was low during February 2003 (9.1 °C), gradually increasing after March (13.6 °C) until reaching unusually high values in June (22.6 °C) and July (22.9 °C). Precipitation was high in April (72 mm), abundant during February (60 mm) and March (51 mm), but very little from May (8 mm) to July (0 mm).

The field was a cultivated area of 1 ha of primary alluvium soil (AL type). Systematic sugarbeet cultivation had started about 28 years previously, with a rotation length of five years. Agricultural practices before and during the study are summarized in Table I.

**Soil sampling for nematodes.** Sampling was performed monthly from February to July 2003. The field was di-
vided into five equal areas. Nematode community analyses were made on the five areas, under the assumption that all plots were uniform regarding soil physico-chemical characteristics. For each area, a "composite sample" was obtained from fifteen sample points, collected from two plant rows, at a 0-20 cm depth. The collected soil was mixed thoroughly and eight sub-samples of 200 g each were used for analysis.

Nematodes were extracted by decanting and sieving (25, 60 and 325 mesh sieves), followed by centrifugal flotation (in sugar solution) (Caveness and Jensen, 1955). After extraction, nematodes were preserved in 4% formalin solution (Barker, 1985). Phytoparasitic nematodes were identified to genus level according to Brzeski (1998) and Siddiqi (2000) under an inverted microscope (Olympus® CK30). Nematode abundance was expressed as number of individuals per 200 g of soil.

Soil sampling for physico-chemical parameter determination. Soil used for determining physico-chemical parameters was collected on each of the six sampling dates from three points along the centre of the terrain. Samples were placed individually in plastic bags. Sub-samples from each bag were taken to determine: pH (H₂O); nitrate content (N-NO₃); extractable phosphorus (P₂O₅) and potassium (K₂O); exchangeable cations, calcium (Ca⁺) and magnesium (Mg⁺); organic matter; soil moisture. A thermometer measured soil temperature (20 cm depth), after allowing twenty minutes for equilibration. Analysis of these parameters followed the usually employed methods (Midgley and Torrance, 1978; Thomas, 1982).

Statistical analyses. The data collected were analysed in three stages.

i) To compare the monthly mean abundance for each phytoparasitic genus, the Kruskal-Wallis non-parametric test was applied, followed by multiple comparisons with the Dwass-Steel-Critchlow-Fligner method (Critchlow and Fligner, 1991).

ii) To determine temporal patterns in plant-parasitic nematodes and in soil parameters, two separate principal component analyses (PCA) with data transformation were employed (Manly, 1986; Legendre and Legendre, 1998).

iii) The relationships between plant parasitic nematodes and soil physico-chemical parameters were investigated using corresponded canonical analyses (CCA) (Manly, 1986; Legendre and Legendre, 1998).

To avoid statistical misinterpretations, the last two stages were repeated without the most abundant genus identified in the first analysis.

These multivariate analyses were carried out using the STATISTICA 6.0© and STATSDIRECT 2.4.1© statistical packages.

RESULTS

Soil physico-chemical parameters. Results of the physico-chemical characterization are summarized in Fig. 1A and Fig. 1B. Soil temperature increased progressively throughout the study, from spring to summer (10 °C-27 °C). Soil moisture content was high from February (24.5%) to April (20.8%) due to the drenching of the soil by the heavy precipitation, and increased after May (12.8%) due to irrigation. The pH was maximum at the beginning of the study (7.6), decreased to a minimum in May (6.2), and increased after soil preparation for the tomato crop. The organic matter content (1.4-1.9%) remained the same, especially from May to July. Calcium (8.44-15.72 meq/100 g) and magnesium (0.87-2.09 meq/100 g) concentrations suffered mild oscillations, with the lowest value in June. Phosphorus (95-181 ppm) and potassium (90-169 ppm) concentrations decreased progressively from February to April (minimum
value), increased significantly during May (maximum value) and decreased again until July. Nitrogen concentrations (12-166 ppm) varied in a similar way but with a highest value in June rather than May.

Results of the principal component analysis on soil parameters are shown in Fig. 2. The first two axes represent 75% of the total variation. The first factor (horizontal axis) with 55% of the variability mainly opposes phosphorus and potassium to soil moisture and calcium. The second factor with 20% of the variability mainly isolates magnesium. The remaining parameters occupy intermediate positions.

In the correlation circle, aggregation occurring between components with higher concentrations/values from February until April is evident. The same situation occurs with those parameters that increased from May to July (soil temperature, nitrogen, phosphorus and potassium – right side). In the first case, the group formed by soil moisture and pH is due to increased values after May. The grouping in the second case illustrates the significant boost in potassium and phosphorus concentrations after soil fertilisation in May, the steady rise of soil temperature as the season progressed, and the isolation of nitrogen is a result of the extremely high concentra-
tion in June, also due to fertilisation (Fig. 2).

The seasonal changes in soil parameters were not a steady progression (Fig. 3). Results in April were closer to those in February/March due to an overall lowering of soil parameters (apart from soil temperature) and were very similar between months (i.e. the months during which the land was fallow). The same situation occurred in June when some parameters decrease in value (chemicals depleted due to cultivation of the crop) and tended towards the values observed in the initial months. The high concentrations of a few of the parameters (mainly phosphorus, nitrogen and potassium) during field preparation and the sowing of a new crop, clearly isolated May from June.

The passage of time emphasized the conjunction of distinct soil parameters in different months. Soil physico-chemical characteristics were very dissimilar during the assay, with important contributions from different parameters in each month. The overall contribution was similar during the four months located in the vicinity of the horizontal axis of Fig. 3. In the two remaining months, some parameters had distinctly low values (soil moisture and pH in May) or high values (nitrogen in June) so that these months stand apart from the horizontal axis.

Fig. 1. B. Soil physico-chemical parameters from February to July 2003 (±standard deviation). Different scales.

Fig. 2. Results of PCA on soil parameters from February to July 2003 at a 0-20 cm depth. A. Eigenvalues; B. Correlation circle F1-F2.

Fig. 3. Results of PCA on soil parameters from February to July 2003 at a 0-20 cm depth.
Phytoparasitic nematodes. During this study, ten genera from nine families of phytoparasitic nematodes were identified: Helicotylenchus (Hoplolaimidae, 96% of the total community), Tylenchus (Tylenchidae, 2%), Tylenchorbynychus, Meloidogyne (Meloidogyidae, 1%), Pratylenchus (Pratylenchidae, 1%), Heterodera (Heterodidae, <1%), Meloidogyne (Meloidogyidae, <1%), Tribochorus (Trichodoridae, <1%), Gracilacus (Paratylenchidae, <1%), Criconemella (Criconematidae, <1%). Even with the large discrepancies between genera relative abundance, results of analyses obtained with and without inclusion of the most abundant were very similar. The densities varied month by month. Even with the high numbers of lower percentages for some genera are a consequence of inclusion of the most abundant were very similar. The abundance, results of analyses obtained with and without these two genera refer exclusively to juveniles.

For phytoparasites, there was an evident separation of the numbers of Helicotylenchus (Hoplolaimidae, <1%) and Meloidogyne (Meloidogyidae, <1%). Helicotylenchus (Hoplolaimidae, <1%) was clearly the most abundant genus, with the most abundant genera, Tylenchus and Helicotylenchus, and a group formed by Merlinius, Pratylenchus, Tylenchorbynychus and Meloidogyne, whose abundances were very small or even null in some months.

The correlation matrix of the monthly factors for the phytoparasitic genera (Fig. 6) showed a distinct seasonal effect between February and March (winter), April (beginning of spring), May (end of spring), and June and July (summer). Factor 1 (the horizontal axis) consisted mainly of time variation, showing the seasonal traits of the community, as months are distributed along the axis. Factor 2 separated the months with lower abundance from those with a greater abundance; the end of spring illustrates the first situation and summer the second.

Thus, the communities from each seasonal group show significant differences, and mainly between winter and summer. The beginning and end of spring had intermediate positions, although the first was close to the winter observations and the second was distinct from all others.

Relationships between phytoparasitic nematodes and soil physico-chemical parameters. The eigenvalues of the canonical correspondence analysis between phytoparasites and soil parameters show that the first factor takes into account 56% of the total variation and that the second factor was responsible for 42% (Fig. 7). This analysis does not show significant differences when compared with the PCA of soil parameters, with phosphorus, nitrogen, potassium and soil temperature opposed to the other parameters; the relations between individual soil parameters are similar to previously.

When Helicotylenchus was not considered in the analysis, differences in the canonical analysis were almost null; this is to be expected since the PCA on phytoparasites did not reveal major differences. Apart from an inversion along the horizontal axis and a slight difference in the CCA total variation, all the relationships remained the same.

For phytoparasites, there was an evident separation
between those with a downward trend in population density in the final months (*Criconemella*, *Gracilacus*, *Trichodorus* and *Heterodera*) and those with increasing population densities in the same period, with a tighter grouping of the genera in the latter group.

The correlation matrix between phytoparasitic nematodes and soil physico-chemical parameters is summarized in Fig. 8. *Heterodera* showed the greatest number of correlations (five) with the nine parameters, and was highly negatively correlated with soil temperature. *Tri-
Chodorus, Criconemella and Gracilacus had four significant correlations, all with the same four parameters, namely soil temperature, soil moisture, pH and organic matter, and all strongly positively associated with organic matter. The remaining genera had only single positive relationships: Tylenchus, Merlinius, Helicotylenchus and Pratylenchus with pH, Tylenchorhynchus and Meloidogyne with soil temperature. Overall, the positive correlations were in the majority.

Correlations with soil moisture, pH and organic matter were always positive; on the other hand the only one with potassium was negative. The greatest number of correlations were with pH, a total of eight. There were no correlations with phosphorus, nitrogen, calcium or magnesium.

DISCUSSION

The phytoparasitic community. The existence of a high density of phytoparasitic nematodes at the beginning of this study allowed us to postulate their strong presence.
during the previous cropping period and/or the development of a series of adaptations that counteract the absence of a “living” host (Kable and Mai, 1968).

However, the later absence of a developing crop and the network of fungi and soil bacteria usually associated with roots contributed strongly to a lowering of phytoparasite abundance during the fallow period (Weaver et al., 1995), with special relevance in the case of *Heterodera*, usually the main genus responsible for the loss of productivity in sugarbeet fields. Other factors may have contributed to the decline in the phytoparasite population, such as the poor adaptation of these organisms to the natural plant succession that occurs as plant cover moves from crop to various weeds (Donfack et al., 1995); any re-adaptation to a different species of plant host may not be automatic, and in some cases may require a transitory period (Baujard and Martiny, 1995).

After a fallow period, *Criconemella*, *Gracilacus* and *Trichodorus* were no longer found and the abundance of *Heterodera* reached very low values. An opposite situation occurred with *Meloidogyne*; despite a low incidence from February until April, its presence increased after crop introduction. As values obtained for *Heterodera* and for *Meloidogyne* refer exclusively to juveniles, their population densities and potential effects are underestimated.

*Helicotylenchus* was undoubtedly the dominant phytoparasite with monthly abundances of more than 95% of the total community, clearly higher than the values obtained in other crops (corn, soybean) by Cares and Huang (1991), Mattos (1999) and Goulart et al. (2003), where they formed around 40% of the total.

For many years, the farming techniques in this area were based on traditional cropping, followed by a short fallow period and a new crop. The genus *Helicotylenchus* is very adaptable, even when the same crop is grown repeatedly, being able to survive and reproduce in frequently changing environments, even with different food sources (Kandji et al., 2001; Gomes et al., 2003; Goulart et al., 2003). Being an ectoparasite, a damaging effect on the crop only occurs in the presence of a massive number of individuals; in our case, it is certainly possible that *Helicotylenchus* might have had a harmful effect on the new crop.

**Phytoparasitic nematodes and soil parameters.** In sum-
mary, we have demonstrated that there are well defined relationships between phytoparasitic nematodes and soil parameters. Nitrogen, phosphorus and potassium (traditionally incorporated in the fertilization) concentrations were very close in the PCA. When comparing results between PCA on phytoparasites and the correlations established, those for *Trichodorus*, *Criconemella*, *Gracilacus* and *Heterodera* were the most similar, and the negative effect of soil temperature strengthened the separation of these four genera from the others, particularly *Tylenchorhynchus* and *Meloidogyne*.

During this study, clear monthly changes occurred and these reflected the agricultural circumstances. The community structures for February/March and for June/July, this last with greater numbers of individuals, reflect what has occurred in terms of agronomical practices: fallow and cultivation. The other two months were in an intermediate position, being distinguished by the lesser number of individuals in April, given the reduction in soil nutrients, and in May due to land management practices.

*Heterodera*, *Gracilacus*, *Trichodorus* and *Criconemella*, present in greatest numbers at the beginning of the year, displayed an evident affinity with the amount of organic matter, but also with pH and soil moisture; in fact they were the only phytoparasites correlated with the last parameter mentioned. Higher soil temperatures and, to a minor extent, a greater concentration of nitrogen, phosphorus and potassium (from fertilization) after May were clearly adverse for them.

The increasing abundances of *Tylenchorhynchus* and *Meloidogyne* in June and July was influenced by the increasing soil temperature and to a lesser degree by a higher concentration of phosphorus, nitrogen and potassium, which counteracted the antagonistic effect of the pH. *Helicotylenchus*, *Merlinius*, *Tylenchus* and *Pratylenchus* showed a certain preference for pH values and, to a minor extent, soil moisture. No parameter affected them to a significant degree, so their seasonality was more dependent on the host than on the soil.

Crop absence during part of the study may hypothetically create correlations between abundance of some genera and pH (Norton, 1991; Cadet et al., 1994; Korthals et al., 1996), soil moisture (McSorley, 1997; Yeates, 1998) and organic matter (Norton et al., 1971). The positive correlations shown by the great majority of phytoparasitic nematodes with the increasing soil moisture and temperature may have been diluted due to the introduction of a tomato crop in April. Soil moisture was a limiting factor only in April/May since before that period the soil was drenched by rains and afterwards was permanently irrigated. Both situations of greater soil moisture match the highest nematode densities recorded (Ferris et al., 1971; Wallace, 1973; Rickard and Barker, 1982).

*Impact of land management on nematodes. The decline in the number of individuals during the fallow period (February/April) was at the time that a crop able to support phytoparasitic nematodes was absent, and there were no land management practices that would provide soil enrichment in terms of nutrients or water. The following seems to have a noticeable effect on a few genera and to temporarily diminish the abundance of others. According to Yeates (1991), Neher (2001) and Gomes et al. (2003), some nematodes may have a high sensitivity to a specific type of environmental disturbance, declining to undetectable levels within a short period of time.

Seasonal changes with time, characterized by lower soil moisture and increased temperatures, were other factors that may have contributed to the reduced numbers of nematodes at the end of fallow (Gupta and Yeates, 1997). Soil moisture is not the only parameter to determine the densities of phytoparasitic nematodes found, and others, such as the presence of weeds after harvest, residues from the previous crop or nutrients accumulated (mainly nitrogen) by those plants, help to maintain high phytoparasite populations in the periods between crops (Anwar et al., 1992; Wasilewska, 1997).

In May, land management in preparation for the tomato crop, with the subsequent removal of old roots/weeds and introduction of fertilizers and protective chemicals, drastically altered the situation, hastening the decline in nematode abundance (Freckman and Caswell, 1985; Bongers, 1990; Yeates and Hughes, 1990).

In the following months, changes induced by the presence of the crop and the constant irrigation benefited those nematodes that use tomato roots as a food resource (Barker and Campbell, 1981; Ettema and Bongers, 1993). Thus the situation in which phytoparasite densities were decreasing was reversed and their densities increased again.

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**LITERATURE CITED**


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