The Effect of Flue-cured Tobacco Monoculture and Different Types of Crop Rotations on Population Densities of Plant-parasitic Nematodes

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SUMMARY

Phytosanitary function of crop rotation is a known fact, so one of the basic motives for this work was to investigate its influence on the population densities of plantparasitic nematodes. Long-term experiments, which are still in progress, were set up on luvic semigley on multi-layered Pleistocene sands on the experimental field of the Tobacco Institute Zagreb at Pitomača. Along with tobacco monoculture, investigations also included seven different types of crop rotations. As key crop, tobacco is included in all crop rotations. Results obtained indicate that presence of the population of endoparasitic nematodes, genus Pratylenchus, was to some degree crop dependent. Tobacco does not seem to stimulate intensive occurrence of this nematode population. However, as soon as it is included into crop rotation, the number of nematodes increases particularly in crop rotations involving a larger number of rotation fields. Soybean and oil-seed rape favour the spread of Pratylenchus species. Still, no alarming incidence of nematodes belonging to this genus was recorded. Ectoparasitic nematodes involve genera Tylenchus, Tylenchorhynchus and, to some extent, Paratylenchus, while incidence of genera Helicotylenchus and Rotylenchus is negligible. Members of these genera are no dangerous pests for the crops studied, even more so as they mainly appeared in populations that are not noxious. The established opinion about the population of saprophagous nematodes being more dependent on the amount of soil organic matter than on the crop type was not convincingly confirmed in this research.

KEY WORDS

Crop rotations, plant-parasitic nematodes, tobacco monoculture

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Utjecaj monokulture flue-cured duhana i različitih tipova plodoreda na gustoću populacije biljno-parazitskih nematoda

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SAŽETAK

Fitosanitetska funkcija plodoreda poznata je činjenica, pa je jedan od temeljnih povoda za ova istraživanja bio da se istraži njegov utjecaj na populaciju nekih rodova biljno-parazitskih nematoda. Višegodišnja istraživanja, koja se i dalje nastavljaju, provedena su na lesiviranom tlu na višeslojnim pleistocenskim pijescima na pokušalištu Duhanskog instituta Zagreb u Pitomači. U pokusu je, pored monokulture duhana, zastupljeno sedam različitih tipova plodoreda. Kao ključna kultura, duhan je zastupljen u svim plodoredima.

Zastupljenost populacije endoparazitskih nematoda roda *Pratylenchus* do određenog stupnja ovisila je o usjevu. Duhan očito ne potiče jaču pojavu populacije nematoda ovog roda. No, čim se uključi u plodored broj se nematoda povećava, napose u plodoredima s većim brojem plodorednih polja. Soja i uljana repica pogoduju širenju *Pratylenchus* vrsta. Ipak, nematode ovog roda nisu se pojavile u zabrinjavajućem stupnju.

Ectoparazitske nematode zastupljene su rodovima *Tylenchus, Tylenchorhynchus* i, donekle, *Paratylenchus*, a savim beznačajno rodovima *Helicotylenchus* i *Rotylenchus.* Pripadnici ovih rodova nisu opasni štetnici za istraživane kulture, tim više što su najčešće bili zastupljeni u populacijama koje nisu opasne.

Uvriježeno mišljenje da je populacija saprofitskih nematoda ovisnija o količini organske tvari u tlu nego o vrsti usjeva nije u ovim istraživanjima uvjerljivo potvrđeno.

KLJUČNE RIJEČI

biljno-parazitske nematode, monokultura duhana, plodored

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INTRODUCTION

Because of the fact that crop rotation is an agroeconomic category that, among other things, also has a phytosanitary function, we found it useful to investigate, among the other things, its effect on the population densities of certain genera of plant-parasitic nematodes.

Of special importance for us is research done on the spread of these nematodes and the damage they can cause to crops, notably tobacco, in the conditions prevailing in Northern Croatia (Korunović and Oštrec, 1977; Oštrec, 1988). It was determined in this research that plant-parasitic nematode genera *Meloidogyne* and *Pratylenchus* prevail on tobacco fields in Northern Croatia, with the highest population density in summer and autumn periods, and the lowest in spring. Large presence of *Pratylenchus* species on the mentioned tobacco fields is important for our trials, which were conducted in the same tobacco growing region, despite the fact that most harmful to tobacco are the nematodes belonging to genus *Meloidogyne*, which were not the subject of this research. Members of genus *Pratylenchus* are also significant tobacco pests and have been included into the group of most dangerous tobacco parasites in the USA and Canada (Lucas, 1986). Tobacco is not a good host for the development and propagation of *Pratylenchus* species but, according to Lucas (1965) and Milne (1972), it is very susceptible to their attack. According to the results obtained by Olthof et al. (1973), species *Pratylenchus penetrans* caused an 11% decrease of tobacco yield at the population of 6000 nematodes per 1 kg soil, and a 27.5% decrease at the population of 18000 per 1 kg soil.

We also determined ectoparasitic nematodes belonging to genera *Tylenchus*, *Tylenchorhynchus* and *Paratylenchus*, as well as *Helicotylenchus* and *Rotylenchus*, which can, according to Lucas (1965), directly or indirectly damage tobacco as carriers of virus, bacterial and fungal diseases. Genus *Tylenchus* nematodes appear in high populations, but whether they are major tobacco pests is still unknown.

According to Arsenault et al. (1989), Pratylenchus penetrans population was higher and tobacco yield lower when tobacco succeeded soybean than when it was grown after rye in two of the three years, while in one year it was equal in both crop sequences. Analogous results were achieved by Kimpinski et al. (1987). A higher nematode population was recorded in soybean growing, while the nematode population decreased when rye succeeded soybean and preceded tobacco. These authors recommend soil fumigation after soybean if this is succeeded by tobacco. Mukhopadhyaya and Prasad (1969) also investigated the effect of crop rotation upon nematodes as well as their effect on yield. As regards the degree of infection, by e.g. *Pratylenchus* penetrans, and the resulting damage, there are no uniform criteria, but criteria differ for particular crops, which is understandable with respect to their different resistance to this particular nematode. While infection with more than 10 nematodes is classified as severe for

narcissi or 40 for irises, the corresponding values for root plants are 100 to 200 nematodes in 100 ml of soil.

Nusbaum and Ferris (1973) studied the role of cropping systems in controlling populations of plant-parasitic nematodes from different aspects (populations of nematodes and life communities, ecosystem stability, nematode and host plant relationship, epidemiology, crop selection for different crop rotations, nematode population dynamics, ecological and economic consequences and integral control). According to these authors, the community of plant-parasitic nematodes is an integral part of the complex of soil microorganisms forming a vital subsystem within the overall ecosystem. Collins and Rodriguez-Kabana (1971) investigated how the relationship between fertilization and crop sequence affected population of Pratylenchus species. In many cases, crop rotation is regarded as the conventional method of nematode control (Barker, 1991) and it has been accepted by growers because it contributes to improvement of soil fertility and yielding of plants. Communities of plant-parasitic nematodes, as an integral part of the ecosystem, are exposed to environmental factors as well as those influenced by man. Continuous monocropping, lacking both inter- and intra-specific diversity, is the worst agroecosystem complex and, therefore, the least stable one. In contrast to monocropping, crop rotation is one of the options of nematode population regulation, its greatest potential doubtlessly involving the role that it may have in integral protection in the future. It is also expected that crop rotation, as opposed to monocropping, will have a positive influence on the agroecosystem since it increases its diversity and stability. Here, the selection of an adequate crop sequence is essential.

MATERIALS AND METHODS

Problems addressed in this chapter are dealt with in more detail in the other paper (Butorac at al., in press). This paper presents only the parts of research methods that are relevant to the interpretation of the results treated in it. Trials were set up on luvic semigley on multi-layered Pleistocene sands, on the experimental field of the Tobacco Institute Zagreb at Pitomača (Northern Croatia), according to the randomized block design method, with a systematic lay-out of trial plots within blocks. The trial includes seven different crop rotation types as well as tobacco monoculture, as clearly shown in the tables providing investigation results, naturally along with participating crops and their sequence. Unfertilized tobacco monoculture was introduced subsequently, so it covers only some of the indicators. Tobacco is the key crop in the investigations, which are conceived so as to enable an insight into a number of different parameters relating to plant and soil. This paper covers research done on presence of some plant-parasitic nematode populations, all per tobacco monoculture and different crop rotations.

Population densities of plant-parasitic nematodes was determined in soil samples taken and refers to their

number in 100 ml of soil. Soil samples for nematode assay were collected randomly from entire experimental field. Corres collected from each plot were mixed thoroughly. Samples were washed using the sedimentation method or Seinhorst's method (Seinhorst, 1955). This method enables separation of free-living nematodes from the soil. Promptly after separation, nematodes were counted and their genera were determined under binocular magnifying glass (magnification 50 x). The procedure can be extended up to determination of particular species by making permanent preparations, which are examined under a microscope (magnification 100 to 10 000 x). Such enlargement makes it possible to clearly examine particular organs and body parts and, thus, determine the species. In accordance with the set goal, our research on endoparasitic nematodes was restricted to genus Pratylenchus as the only one present, and to ectoparasitic genera Tylenchus, Tylenchorhynchus, Paratylenchus, Helicotylenchus, and Rotylenchus. As the latter two genera were only occasionally recorded and with only slight intensity, values estimated for them are not provided. In addition to endo- and ectoparasitic genera, participation of saprophagous nematodes was also determined.

RESULTS AND DISCUSSION

Endoparasitic nematodes

Crop rotation constitutes one of the oldest and most important approaches to the control of nematodes feeding on the roots of annual crops. Its success depends on the extent to which crop rotation prevents nematode development and reproduction. Crop rotation can be disregarded in the case of efficient nematicide application, or development of nematode-resistant cultivars. These three measures (crop rotation, nematicide application and resistant cultivars) are not mutually exclusive and are aimed at maintaining pedohygiene at a tolerant level.

Genus *Pratylenchus* was present throughout the whole 9-year trial period (Table 1). It seems that changes in the nematode population of this genus appeared as early as in the first year as a result of crop rotation diversity, while anti-nematode action of some crops (fodder radish, cultivar Rauola, Phacelia, maize) is also discernible. Rather variable results were achieved in the second year, both with regard to crop species and crop rotation types, as well as seasonal fluctuation. Namely, nematode population in most crops is the lowest in the autumn term. Genus Pratylenchus population under tobacco, the key trial crop, varies in dependence on crop rotation, as well as on the preceding crop (winter wheat or oil-seed rape). Tobacco monocropping itself did not affect the population densities of nematodes. Moreover, their number was generally decreased relative to the preceding year. Presence of nematodes, members of genus Pratylenchus, under wheat obviously depends on the preceding crop and crop rotation type. The highest nematode presence was recorded, as a rule, in crop rotations in which winter wheat was preceded by soybean. Soybean greatly contributes to the spread of genus *Pratylenchus* nematodes throughout the growing season, which was also noted by other authors, regardless of its being preceded by maize, known for its anti-nematode action, in all crop rotations. Similar soybean bearing persisted more or less throughout the whole trial period.

In the third year, population of genus *Pratylenchus* nematodes was relatively variable per crops and crop rotation types. For instance, its presence in tobacco monoculture was very low, whereas it was much higher under tobacco grown after oil-seed rape, and under oil-seed rape itself, as well as in six-year rotation under soybean in the autumn term. It would be certainly wrong to consider the population of genus *Pratylenchus* nematodes only through crop rotations and their respective crops without taking into account the prevailing meteorological conditions, as well as the root system development. Intensified root development causes nematodes to pass from the soil into roots, thus decreasing their population in the soil.

Fourth year results also indicate that tobacco grown in monoculture is not a good host plant for genus *Pratylenchus* species though, according to literature data, it is susceptible to their attack. However, tobacco growing in crop rotation with other crops makes the situation worse, especially if oil-seed rape and soybean are included in crop rotation.

In the fifth year, population of genus *Pratylenchus* nematodes was generally more numerous than in preceding years, which might specifically apply to most crop rotations and their respective crops in the autumn term. It was particularly high under oil-seed rape, as well as under soybean, but even much higher under tobacco, which was generally included into crop rotations stretching over a larger number of years.

It is noteworthy that the soil of the trial area is rather poor in nematode fauna, which is strongly corroborated by the presence of only four nematode genera. This is the reason why, also in the sixth year, only one genus of endoparasitic nematodes is discussed, viz. *Pratylenchus* species, the dynamics of which varies per seasons regardless of crop rotation types and crops. As a rule, with negligible exceptions, their number rises from spring towards autumn, which is most probably related to hydrothermal characteristics of the climate, as well as to other mediating factors. Population of genus *Pratylenchus* nematodes estimated for this year is perhaps the best example, implying that the final evaluation of the achieved results requires a more detailed analysis of factors such as crop position in crop rotation, crop species and rotation value, particularly its anti-nematode action, crop rotation type, soil properties and hydrothermal characteristics of climate.

In comparison with the preceding year, when, like in the two succeeding years, the investigation programme was restricted due to lack of funds (population of genus *Pratylenchus* nematodes was followed only during one term), the obtained results show that, in the seventh year, no nematodes appeared in 16 out of possible 27 cases. Their presence was slightly higher only under soybean in 5-year rotation and in maize in 6-year rotation. For the sake of objectiveness, it could be said that one determination cannot give a real picture of the state prevailing throughout the whole growing season, which also applies to the succeeding two years. This was confirmed by the experience from earlier years, but it also indicates an improved state with regard to nematodes in this year.

In the eighth trial year, though slightly more numerous, nematode population was not alarming, excepting partly oil-seed rape and soybean.

In the last, ninth year, the genus *Pratylenchus* nematode population appeared continuously under all crops in 4-year B and 5-year rotations, however not with high presence, while in other crop rotations only sporadically.

Average values for the nine-year period reflect, in a way, the described state per particular years. Tobacco is obviously a crop that does not stimulate higher incidence of the genus *Pratylenchus* nematode population. However, once it is incorporated into a crop rotation, the nematode number increases, particularly in crop rotations involving a larger number of years. A similar pattern was recorded for winter wheat as well. Except in 6-year rotation, maize behaved equally in all crop rotations. The more numerous nematode population was probably caused by a higher participation of legumes, primarily soybean. Soybean obviously favours spreading of *Pratylenchus* species, much more than red clover, which is in this respect surpassed by oil-seed rape.

To conclude, regardless of tobacco monocropping and particular types of crop rotations, their physiognomy and seasonal and annual fluctuations, it can be said that genus *Pratylenchus* nematode populations did not appear in alarming numbers. Of special significance is the fact that tobacco itself does not favour their spread but that oil-seed rape and soybean may greatly contribute to it.

Ectoparasitic nematodes

As already mentioned, ectoparasitic nematodes were represented by genera *Tylenchus* and *Tylenchorhyncus* (Tables 2 and 3), as well as by genus *Paratylenchus*. Members of these genera are pests not dangerous for the crops included in the tested crop rotations, even more so as they generally appeared in populations that are not noxious. During the entire trial period, the highest presence recorded was that of genus *Tylenchus* and then genus *Tylenchorhyncus* populations, while genus *Pratylenchus* participation was negligible.

Ectoparasitic genera *Tylenchus* and *Tylenchorhyncus* otherwise extend over a broad range, they are polyphagous, but not very harmful. Species belonging

to these genera are typical secondary parasites. Their populations were not alarming even in crop rotations and in years in which these nematodes were more numerous.

Based on the obtained results, it cannot be maintained certainty that genera Tylenchus and with Tylenchorhyncus species were steadily associated with a certain season. Rather variable results point more to their exposure to changeable weather conditions, the influence of which was indirectly reflected through the soil. Hence, high fluctuations are present, both per seasons and years, but also per crops within different crop rotations. Omitting a more detailed analysis, it can be stated that, according to the 9-year average values, the population of genus Tylenchus nematodes under tobacco was most numerous in 4-year rotation B and least numerous in 6-year rotation. It was about equal in both two-year rotations, equal in 3-year and 5-year rotations, and slightly higher in four-year rotation A. Nematode population of this genus was rather variable under wheat, showing a marked downward trend in multi-year crop rotations. It was lower under maize than under wheat, ranging at about the same level under soybean. It was slightly higher under oil-seed rape in five-year rotation and among the lowest in the trial under red clover.

According to 9-year mean values, population of genus *Tylenchorhyncus* nematodes was considerably below that of the preceding genus and, as a rule, most numerous under wheat in all crop rotations, *per se*, then under red clover, soybean and oil-seed rape, more numerous in multi-year than in other crop rotations. Values under maize vary in dependence on crop rotation, while the values under tobacco are among the lowest in the trial.

Genus *Paratylenchus* species were not recorded in some seasons, not even in a considerable number of years. Still, they were on average most numerous under tobacco monoculture (18), slightly more numerous under tobacco grown in crop rotation, e.g. 15 in three-year or 10 in six-year rotations, and at the same level under soybean and red clover.

Nematode populations of genera *Helicotylenchus* and *Rotylenchus* appeared with only a few exemplars during the trial period, which indicates that the soil was poor in nematode fauna.

Due to space restrictions, tables do not include data on populations of saprophagous nematodes, which were determined with the same frequency as the mentioned genera of endo- and ectoparasitic nematodes. According to some authors, presence of free-living saprophagous nematodes is often more dependent on the amount of soil organic matter than on crop type. Our 9-year average values for particular crop rotations and their respective crops only partially corroborate this opinion. Namely, the highest population of saprophagous nematodes was recorded in two-year rotation A and ranged from 564 under winter wheat and green manure

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80 120 40 80 0 60 40 60 80 70 0 60 60 140 60 180 20 60 30 80 40 340 20 120 50 20 0 0 90 310 10 40 30 40 0 0 40 340 20 120 30 40 0 0 140 60 140 60 30 40 0 0 120 120 120 20 50 60 0 130 70 180 20 50 60 0 120 120 120 80 40 0 120 110 40 120 80 40 0 120 120 120 80 40 0 120 120 120 140 60 140 0 120 120 140 0 120 120 120 120 140 0 120 120 120 120 140 0 120 10 0 120 </td <td>Tobacco</td> <td>180</td> <td>60</td> <td>80</td> <td>0</td> <td>180</td> <td>100</td> <td>90</td> <td>120</td> <td>180</td> <td>200</td> <td>06</td> <td>260</td> <td>320</td> <td>170</td> <td>80</td> <td>180</td> <td>0</td> <td>160</td> <td>40</td> <td>131</td>	Tobacco	180	60	80	0	180	100	90	120	180	200	06	260	320	170	80	180	0	160	40	131
80 70 0 60 60 140 60 180 20 60 30 80 40 340 20 120 50 20 0 0 90 310 10 40 30 40 0 0 90 310 10 40 30 40 0 0 140 60 140 60 30 40 0 0 130 70 180 20 50 60 0 120 120 120 80 40 0 120 110 40 120 20 50 60 0 120 120 120 80 40 0 120 110 40 120 80 40 0 120 110 40 120 80 40 0 0 120 120 120 140 6 0 140 0 120 120 20 0 0 0 0 120 120 140 0 120 10 0 120 140 0 0 1	Maize	80	120	40	80	0	60	40	60	70	160	30	260	160	06	210	420	40	50	40	106
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50 20 0 0 90 310 10 40 40 80 40 40 0 60 140 60 30 40 0 0 40 90 310 12 40 20 50 60 0 0 130 70 180 60 0 0 120 110 40 120 120 80 40 70 0 30 20 0 120 120 20 50 0 0 120 110 40 120 120 20 0 0 0 120 110 40 120 20 0 0 0 120 0 120 120 40 40 10 0 140 0 120 120 20 0 0 0 140 0 120 120 40 0 0 140 0 120 120 120 140	Oil-seed rape	20	60	30	80	40	340	20	120	180	0	120	180	180	50	130	120	20	50	170	100
40 80 40 40 0 60 140 60 30 40 0 0 40 90 120 120 20 50 60 0 0 130 70 180 60 0 0 120 110 40 120 80 40 70 0 30 20 0 120 20 50 30 0 20 0 120 120 40 40 70 0 0 10 120 120 140 40 10 0 140 0 120 140 40 140 0 120 120 140 40 140 0 120 120	Tobacco	50	20	0	0	06	310	10	40	260	90	110	140	220	06	60	120	120	120	06	102
30 40 0 0 40 90 120 120 20 50 60 0 0 130 70 180 60 0 0 0 120 110 40 120 80 40 70 0 30 20 0 120 20 50 30 0 20 0 120 40 4 0 0 140 0 120 140 4 0 0 140 0 120	Maize	40	80	40	40	0	60	140	60	100	30	130	220	280	150	120	300	0	40	40	98
20 50 60 0 0 130 70 180 60 0 0 0 120 110 40 120 80 40 70 0 30 20 0 120 20 50 30 0 20 0 120 120 40 4 0 0 120 0 120 120 20 50 30 0 20 0 120 120 40 4 0 0 140 0 120 120 140 4 0 0 140 0 120 120	Soybean	30	40	0	0	40	06	120	120	120	70	120	320	200	60	100	210	0	50	120	95
Anheat 20 50 60 0 0 130 70 180 er 60 0 0 0 120 110 40 120 er 80 40 70 0 30 20 0 120 er 20 50 30 0 20 0 120 er 20 50 30 0 20 0 120 100 40 4 0 0 120 120	Six-year rotation																				
Er 60 0 0 120 110 40 120 er 80 40 70 0 30 20 0 120 er 20 50 30 0 20 0 120 er 20 50 30 0 20 0 120 140 4 0 0 140 0 120	Winter wheat	20	50	60	0	0	130	70	180	06	70	50	120	80	110	140	200	20	80	06	82
er 80 40 70 0 30 20 0 120 er 20 50 30 0 20 0 120 40 4 0 0 40 140 0 120	Tobacco	60	0	0	0	120	110	40	120	160	60	70	110	240	90	260	80	0	90	50	87
er 20 50 30 0 20 0 0 120 40 4 0 0 40 140 0 120 140 40 0 130 0 140 0 120	Red clover	80	40	70	0	30	20	0	120	60	120	20	160	220	80	320	06	0	70	0	79
40 4 0 0 40 140 0 120	Red clover	20	50	30	0	20	0	0	120	100	160	20	120	240	20	80	110	20	210	10	70
	Maize	40	4	0	0	40	140	0	120	0	30	160	240	160	80	20	210	20	30	80	72
	Soybean	140	40	0	120	0	140	0	180	70	40	50	120	120	60	110	100	0	40	110	76

Table 2. Population of ecto-parasitic *Tylenchus spp.* nematodes

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0 0 0 0 0 0 0

6 6 8 0 90 20 6 6 8 0 9 0 90 0

0 0 4 0 0 0

0 0

Winter wheat

Tobacco

Red clover Red clover

Soybean

Maize

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crop to 612 under tobacco. It was lower in other crop rotations, without green manure, amounting to only 354 nematodes in five-year rotation. In principle, it was lower under tobacco in crop rotations extending over a larger number of rotation fields than in crop rotations of fewer fields. As regards crops themselves, populations of saprophagous nematodes were most numerous, except for the stated value for tobacco, under winter wheat, and again somewhat less numerous in multi-year rotations. This was followed by oil-seed rape, then soybean and red clover, while maize came last.

Since communities of plant-parasitic nematodes, as an integral part of the ecosystem, change under the influence of ecological factors, including anthropogenic impact, it along these lines that also our research results should be considered. Roots and the rhizosphere of sustainable host plants encompass various ecological niches, in which plant-parasitic nematodes develop and reproduce. Food supply is, therefore, one of the major determinants in seasonal and annual changes of the population structure, density and spread, which is in a way corroborated by our results on populations of endoand ectoparasitic nematode genera Pratylenchus, Tylenchus and Tylenehorhynchus. We support the statement that the determined seasonal and annual fluctuations, but also fluctuations within particular crop rotations, is a consequence of the above apostrophized factors, viz. different ecological niches as well as different availability and different sources of food. It may be assumed that endo- and ectoparasitic nematodes feeding on the same root occupy spacially separated niches but still effect one another because both groups depend on the same source of food. Although interaction mechanisms and ecological significance of association of nematode species are not guite clear, the practiced systems of plant production, and more closely the practiced crop rotations within them, largely determine the structure of communities in which they appear.

Lack of diversity in nematode fauna determined in our trials is probably related to the strong anthropogenic influence on the soil because, as soon as crop growing is discontinued, arable soil returns to its natural climax and pest communities become more complex and more stable.

Effect of crops on the nematode population density is also influenced by the length of exposure to their attack. Our results show that no more severe attack of either endo- or ectoparasitic nematodes occurred, which could endanger the crops grown. This might be partly explained by the fact that attempts were made to sow crops before the onset of conditions favouring nematode activity. Although ecological conditions have a direct influence on nematode populations, perhaps of even greater importance are the indirect effects of host plants, naturally along with measures such as growing of multiline cultivars, crop rotation, changing of cultivars or growing of alternative crops.

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