PhD thesis

Environmentally friendly control methods against the southern root-knot nematode (*Meloidogyne incognita* CHITWOOD) in forced vegetables

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I. INTRODUCTION AND RESEARCH OBJECTIVES

In the case of soil-grown vegetables, the most significant plant health problems of forcing are the pests and diseases present in the soil. Namely, the southern root-knot nematode, *Meloidogyne incognita* (KOFOID and WHITE, 1919) CHITWOOD, 1949, which is the most common pest nematode species in Hungarian greenhouses, is one of the major pests. It may cause severe damage to most vegetables in forcing (Dabaj et al., 1994; Amin, 1994). *Meloidogyne incognita* is an introduced species of tropical origin, which may get into outdoor soils primarily by infested transplants (Andrássy and Farkas, 1988). Nevertheless, overwintering of the species under open field conditions was also observed during the mild winters of the past few years in Hungary (Budai et al., 2005). Control of root-knot nematodes is inevitable so as to make vegetable production profitable, thus developing new and environmentally friendly control methods is required. The use of resistant cultivars as well as grafting onto resistant rootstocks might be a pesticide-free, environmentally safe and also effective solution for the problem.

Pepper is the most important forced vegetable in Hungary having a growing area of approx. 1520 ha (Anonymus, 2011). Up to date, nematode resistance genes in the cases of several wild and cultivated species of the family *Solanaceae* have been identified, so grafting and developing resistant cultivars might be effective and environmentally safe alternative ways of nematode control in pepper forcing, too (Thies et Fery, 2003; Oka et al., 2004). Though grafting is an expensive process, it might be a solution until an appropriate selection of resistant cultivars is placed on the market. That is why breeders' work should be helped so that a wide range of root-knot nematode resistant cultivars may become available to the Hungarian growers as soon as possible.

Because of the extreme weather conditions of the recent years, more and more producers decide to grow plants in greenhouses instead of growing under open field conditions. Greater crop safety and an intensive technology might be provided, and, therefore, higher yields might be achieved in protected cultivation contrary to outdoor production, which is more exposed to weather adversities. For the past few years, the growing area of forced melon has increased, so growers increasingly have to face the damage by root-knot nematodes that require high temperature for their development and occure in large numbers in the soils of greenhouses even there where melon is grown. As melon cultivars resistant to *Meloidogyne* species are not yet available, grafting onto resistant rootstocks, similarly to cucumber production, migh offer an efficient and environmentally safe solution for root-knot nematode control. Because of the general plant protection problems related to root-knot nematodes in greenhouses, investigation on currently used and potential rootstocks for melon concerning their resistance to *Meloidogyne incognita* is proposed. As the

warming climate calls our attention to the more frequently occurring risk of successful overwintering of introduced *Meloidogyne* species outdoors, and as watermelon is a favourable host plant of *M. incognita*, the assessment of root-knot nematode resistance of rootstocks for watermelon is also an important task.

Based on these, my research objectives were the following:

- Testing the resistance of pepper breeding lines to *Meloidogyne incognita* to help the further work of the breeders
- Studying the possibility of environmentally friendly *M. incognita* control in cold forcing by using pepper cultivars grafted onto resistant rootstocks and by using new breeding lines
- Examining the effect of the character of resistance to *M. incognita* on the occurrence of calcium deficiency symptoms on fruits in pepper
- Investigation on the currently used and further potential rootstocks for melon concerning their resistance to *M. incognita*.

II. MATERIALS AND METHODS

Identification of the root-knot nematode species used in the experiments

Taking into consideration the fact that the resistance of plant species to *Meloidogyne* spp. depends on the nematode species, breeding for resistance might only be successful if the stock culture or population of nematodes used for testing resistance consists of specimens all belonging to the same species identified properly. Therefore, the root-knot nematode species that was used for the infestation of different vegetable species and cultivars to test their resistance to the pest was previously identified in each case. The identification was carried out by examining the morphological characters of the vulva, and, after that, PCR technology was used for confirmation. The latter work was helped by Szabolcs Ruthner (Department of Genetics and Plant Breeding, Corvinus University of Budapest).

Testing for resistance

a) Testing the resistance of pepper cultivars and breeding lines to *Meloidogyne incognita*

Between 2003 and 2008, four different experiments were carried out in which totally 80 different pepper cultivars and breeding lines were tested for their resistance to *Meloidogyne incognita*. The studied cultivars and breeding lines belonged to the following species: *Capsicum annuum* L., *C. chinense* Jacq. and *C. bacchatum* L. var. *pendulum*.

Experiment 1: Seeds of the pepper items were sown in closed-cell extruded polystyrene foam trays containing sterile pit sand. Each item consisted

of 10 plants. The seedlings were artificially infested with *M. incognita* at the two true leaf stage on 18. 11. 2003. The nematodes for infestation originated from an unheated large air-space plastic tunnel at Röszke, where pepper cultivar 'Blondy' was grown. The egg sac of the female southern root-knot nematodes was removed from the pepper roots by using a dissecting needle, the eggs of the egg sacs were put in tap water so that they hatch, and finally approx. 800 invasive larvae per plant were injected to the base of the tested plants by using a micropipette. The plants were grown in the phytotron of the Research and Experimental Farm of Soroksár (Faculty of Horticultural Science, Corvinus University of Budapest) at a temperature adjusted to 25–30ºC. The assessment of the extent of damage by examining 6–7 plants per item took place 9 weeks after the artificial infestation.

Experiment 2: Seeds of the pepper items were sown in soil originating from the aforementioned plastic tunnel at Röszke. This soil was severely infested with *M. incognita*. The emerged plants were thinned at the two true leaf stage to have only 10 plants per item. The temperature of the phytotron was adjusted to 25–30ºC. The assessment took place 12 weeks after sowing.

Experiment 3: The cultivars and new breeding lines that turned out to be resistant during Exp. 1 and Exp. 2 were tested in a continuously used large air-space plastic tunnel whose soil, originating from Röszke, was severely infested with *M. incognita*. The material of infestation of the previous studies originated from this plastic tunnel. Seeds of the cultivars to be tested were sown in closed-cell extruded polystyrene foam trays containing sterile sand in the phytotron of the Research and Experimental Farm of Soroksár (Faculty of Horticultural Science, CUB). When reaching the 4-6 true leaf stage on 14. 07. 2004., twelve plants per item were transplanted in the infested soil of the plastic tunnel.

Cultivar 'HRF' served as a control in each experiment mentioned above.

Experiment 4: Five new experimental rootstock cultivars and two blocky type experimental cultivars were studied concerning their resistance to the southern root-knot nematode. Pepper cultivar 'Kaméleon' served as a control. The seeds were sown in 9 cm pots containing uninfested medium. When reaching the two true leaf stage, the plants were transplanted in 15 cm pots containing soil severely infested with *Meloidogyne incognita*. This soil originated from a greenhouse at Soroksár, where tomato was grown, and served as a material for infestation, too. There were ten replicates per item in this experiment.

In the case of each experiment, the assessment of the extent of damage was carried out by counting the number of galls and egg sacs on the roots of the plants. For this purpose, the roots had previously been cleaned from ground, and a stereomicroscope was used for the examination. In Exp. 4, the weight of the roots was measured with an accuracy of the tenth of a gram, and the number of galls per a unit of root weight was also determined. Statistical analyses were

carried out by using these data. (The Games–Howell test was used in Experiments 1, 2 and 3, and the Kruskal–Wallis and the Mann–Whitney tests were used in the case of Exp. 4.)

b) Testing the resistance of potential rootstocks for melon to *Meloidogyne incognita*

The resistance of species and cultivars belonging to the family Cucurbitaceae to *M. incognita* was studied in two experiments.

The first experiment was carried out in a greenhouse at the Research and Experimental Farm of Soroksár, Corvinus University of Budapest, in the summer period of 2006. Fifteen possible rootstocks for melon were investigated. The interspecific cultivars were the hybrids of *Cucurbita maxima* and *Cucurbita moschata*. Melon cultivar 'Donatello' served as a control. On 07. 07. 2006., the seeds were sown in closed-cell extruded polystyrene foam trays having 4x4 cm cells and containing soil infested with southern root-knot nematodes. The infested soil originated from an unheated plastic tunnel at Röszke, where tomato was grown. Twelve plants per cultivar were transplanted at the two true leaf stage in 14 cm pots containing a soil mixture infested with nematodes. The assessment started from 11. 09. 2006., 8 weeks after potting. The weight of the roots was measured, and the galls formed on the roots and the female nematodes inside the galls were counted by using a stereomicroscope. Statistical analysis was carried out by using the Games–Howell test.

In 2008, thirteen cultivars of eight different species belonging to the family Cucurbitaceae were studied concerning their susceptibility to the southern root-knot nematode. In the case of melon, cultivar 'Donatello', while in the case of watermelon, cultivar 'Chrimson Sweet' served as a control. On 08. 07. 2008., the seeds were sown in trays having 4x4 cm cells and containing peat-based soil mixture. Three weeks later, the emerged plants were transplanted in 14 cm pots containing soil heavily infested with *Meloidogyne incognita*. This soil originated from an unheated greenhouse at Soroksár. The plants were grown in a glasshouse of the Arboretum of Buda, Corvinus University of Budapest. The extent of damage in the cases of the roots of 10–12 plants per cultivar was assessed from 30. 09. 2008. The roots had previously been cleaned from ground, the weight of the roots was measured with an accuracy of the tenth of a gram, and the number of galls formed was counted by using a stereomicroscope. The large galls were split with a dissecting needle, and each female specimen inside these galls was counted. The number of galls per a gram of root was also determined. Statistical analysis was carried out by using the Games–Howell test.

Studies on pepper cultivars grafted onto *Meloidogyne incognita* resistant rootstocks and on non-grafted resistant pepper cultivars in cold forcing

Root-knot nematode resistant cultivars and candidate varieties and pepper cultivars grafted onto root-knot nematode resistant rootstocks were studied in three vegetation periods. All the tested cultivars belonged to the sweet, white-fleshed group of pepper cultivars.

These experiments were carried out in a private producer's unheated plastic tunnel at Soroksár. Openings for ventilation were located at the ends of the 50 m long and 6 m wide greenhouse.

Two rootstocks, which were found to be resistant to *M. incognita* by the owners of the cultivars and according to our earlier results, were used for grafting. In order to obtain plants being in the same developmental stage by the time of transplanting, the seeds of the rootstocks and scions to be grafted were sown in trays having 4x4 cm cells and containing peat-based soil mixture about twenty days earlier than those of the non-grafted cultivars were. Grafting took place at the Experimental Station of Ócsa (Syngenta Seeds Ltd.). The plants were placed in double rows, where the row spacing was 100+50 cm, and the plant-to-plant distance was 30 cm within rows. In 2008, there were sixteen replicates per cultivar or rootstock-scion combination, while, in 2009 and 2010, there were seventeen replicates per cultivar or rootstock-scion combination. The plants were grown with two stems, regularly pruned, and one fruit was left on every main stem and lateral shoot. The temperature data were recorded every 15 minutes by using a TinyTag TGP-4510 automatic air and soil thermometer. In 2008, micro nozzles were used for irrigation, while, in 2009 and 2010, trickle irrigation was performed. The plants were fertigated according to the phenological stage. Picking took place approx. every second week from June until the frosts. During each picking, fruits were collected per plant, and they were separated accordingly. The weight of each fruit was measured, and the total number of fruits per plant was also counted. The fruits were classified according to the weight instead of the size. Statistical analysis was carried out according to the number of the fruits exceeding 90 g in weight and the total weight of these fruits. It was found that a fruit of 90 g in weight of the studied cultivars fitted a size with a fruit diameter of more than 6 cm at its base and a fruit length of more than 10 cm. By using the program SPSS, the data were statistically analysed with one-way ANOVA (after testing of normality and the homogeneity of variance), and the significant differencies were determined with the Tukey–Kramer test. If the variances were not homogeneous, the Games–Howell test was used.

In the cases of the studies on grafted peppers in 2009 and 2010, the number of fruits showing the symptoms of blossom-end rot (BER) was also recorded during each picking. The fruits showing the symptoms of calcium deficiency were collected per plant, and they were separated accordingly. The number of fruits with BER and the rate of damaged fruits were determined. In 2009 and 2010, 45 g/m^2 and 29 g/m^2 calcium oxide (CaO) were used for

fertigation, respectively. At the end of the vegetation periods, the extent of infestation with root-knot nematodes was evaluated in the following ways: the number of galls per a unit of root weight (2009); the root surface covered with galls related to the total root surface (%) (2010). The obtained data were analysed with the Mann–Whitney U-test.

III. RESULTS

Testing for resistance

a) The resistance of pepper cultivars and breeding lines to *Meloidogyne incognita*

According to my results, the pepper cultivars and materials of breeding vary in the degree to which they are susceptible to the southern root-knot nematode (*M. incognita*). Several resistant breeding lines have been found. The results of the artificial infestation have been confirmed by the results of both the studies on sowing in infested soil and transplanting in the infested soil of the plastic tunnel. In comparison, the number of galls formed in the case of the experiment where the seeds were sown in the infested soil considerably exceeded that of the artificial infestation. Based on this result, it is probable that the root-knot nematodes occurred in larger numbers in the soil infested naturally than in the sand infested artificially, and the soil temperature might also exceed 28ºC as established to be critical by Thies and Fery (1998). The pepper plants grown in the infested soil were less developed and smaller than those that were grown in the sterile sand. The reason for this might be that the possibility of becoming infested with nematodes existed in the former case as early as the time of germination. This calls our attention to the importance of plant hygiene and producing healthy planting material. As the results of the three different experiments coincided, the described methods of the artificial infestation using sterile sand, the sowing and the transplanting in infested soil can all be recommended for testing for susceptibility to root-knot nematodes provided the species identification in the case of the nematode population in question has been carried out properly.

Further research is needed on the studied breeding lines concerning their suitability for using them as rootstocks and also their interaction with the scion. The breeding lines which are resistant to root-knot nematodes have to be characterized with several other important properties, too, so that they could become commercially available certified cultivars. However, based on our results, many pepper cultivars having different fruit types and being resistant to root-knot nematodes that require high temperature for their development may become available to the growers in the near future.

b) The resistance of potential rootstocks for melon to *Meloidogyne incognita*

According to the results of the two experiments, the potential rootstocks for melon varied in the degree to which they were susceptible to *Meloidogyne incognita*, however, resistant items have not been found. On the basis of the number of galls, the interspecific (*Cucurbita maxima* X *Cucurbita moschata*) hybrids turned out to be the most susceptibe to *M. incognita* in both experiments. In respect of susceptibility, the melon cultivar 'Donatello' used as a control was not significantly different from them, and it was also severely damaged. The susceptibility of the *Lagenaria* rootstock cultivars was not significantly different compared to that of the melon cultivar that served as a control; however, significantly smaller numbers of galls were formed on them than on the most susceptible interspecific cultivars. The number of galls formed on the roots of *Sycios angulatus* and *Cucurbita ficifolia* was significantly smaller than that found in the case of the cultivar used as a control.

In both experiments, the largest root weight was measured in the cases of the *Lagenaria* species, and, in this respect, the differences were not significant when comparing them to most of the interspecific cultivars tested. The root weight of the melon cultivar that served as a control was significantly smaller in comparison with the root weight of the interspecific and the *Lagenaria* rootstock cultivars, but its root weight was not significantly different either from that of the tested cultivars of *Cucurbita maxima*, *C. ficifolia* and *S. angulatus* or the *Citrullus lanatus* rootstock cultivar 'Oyakkio'. The smallest root weight was measured in the cases of the watermelon cultivars.

Concerning the number of galls per a unit of root weight, the results show that the interspecific cultivars used as rootstocks for melon and the cultivar used as a control were equally susceptible to the southern root-knot nematode. Moreover, more galls were formed on the former cultivars than on the cultivar 'Donatello'. Therefore, the use of the interspecific cultivars as rootstocks can not be recommended if the soil is infested with root-knot nematodes. I found neither *S. angulatus* nor *C. metuliferus* to be absolutely resistant to the pest. However, as they proved to be significantly more resistant compared to most of the susceptible interspecific cultivars, their testing as rootstocks in melon production is proposed. The watermelon cultivars and the *Lagenaria* cultivars used as rootstocks were not significantly different from each other in respect of susceptibility, and the smallest number of galls per 1 g root was formed on the rootstock cultivar 'NUN 3001 RT'. In spite of this fact, the *Lagenaria* cultivars might be able to tolerate the damage due to their large root weight. The roots of the watermelon cultivars were heavily infested. On the basis of the number of galls per a unit of root weight, I found that watermelon and melon are similarly favoured host plants of *Meloidogyne incognita*. Based on the results of the

studies on watermelon cultivars, it can be concluded that the assessment of resistance to the root-knot nematode might be carried out more precisely by taking the number of galls per a unit of root weight into consideration instead of counting the total number of galls per root. No literature has been found on the susceptibility of *Echinocystis lobata* (wild cucumber). According to my results, it was infested to a similar extent to *S. angulatus*, which is used as a rootstock for cucumber for the control of *Meloidogyne incognita*. Therefore, investigation on its compatibility with scions is proposed to carry out.

The role of resistance properties in the control of *Meloidogyne incognita* in pepper forcing

a) The effects of using pepper cultivars resistant to *Meloidogyne incognita* and those of grafting onto resistant rootstocks on pepper yield and quality

The grafted and non-grafted plants, as the materials for transplanting, were in the same developmental stage in each year (2008, 2009 and 2010) of the study. In order to obtain plants being in the same developmental stage by the time of transplanting, the seeds of the rootstocks and scions to be grafted have to be sown about three weeks earlier than those of the non-grafted cultivars have to be.

At the end of the vegetation period in 2008, the roots of each of the susceptible cultivars were severely damaged (the whole root surface was covered with galls). In the case of the newly bred resistant cultivar 'Cinema', the roots of 5 plants among the studied 17 plants were slightly covered with egg sacs (at a rate of 15–20%), while the roots of the other 12 plants were not damaged. In 2009, the roots were studied on the basis of the number of galls formed on them, and I found that the susceptible non-grafted cultivars were moderately infested. The resistant cultivar 'Cinema' showed good resistance to *M. incognita*, and it was only slightly infested. In 2010, the extent of damage observed in the cases of the non-grafted peper cultivars was similar to that which had been found in the previous year. The rootstock cultivars were not damaged in either year.

In 2008, the results of the statistical analysis (ANOVA) concerning all the parameters of fruits measured showed that there were significant differencies in the cases of the studied cultivars and rootstock-scion combinations. In respect of both the weight and number of fruits, the cultivar 'Cinema', which is resistant to *M. incognita*, significantly exceeded the susceptible cultivars. Although the number of fruits in the case of the other resistant experimental cultivar was not significantly smaller than that of 'Cinema', the fruit weight was considerably smaller. In this case, the size of the fruits often remained small in spite of the continuous thinning of fruits. Though varying in the degree, the pepper cultivars

grafted onto resistant rootstocks always exceeded the susceptible cultivars concerning the total weight of fruits. However, these differences did not prove to be significant in all cases. For example, compared to the non-grafted plants, the difference was not significant either in the case of ’Cibere’ or ’Citera’ grafted onto the rootstock ’Robusto’ (P=0.111, and P=0.108) in spite of the fact that the total weight of fruits was higher by 30% and 36%, respectively, in favour of the grafted plants. The cultivar ’Citera’ grafted onto the rootstock ’Snooker’ produced significantly more yield. Compared to the non-grafted plants, the cultivar ’Citera’produced significantly more fruits in total if it was grafted onto either of the rootstocks used; however, this was not the case with the cultivar ’Cibere’. In respect of the total number of fruits, comparing the grafted plants to the resistant cultivar ’Cinema’, the differences were not significant (in the cases of the cultivar ’Cibere’ grafted onto the rootstock ’Robusto’ and the cultivar ’Citera’ grafted onto the rootstock ’Snooker’).

Concerning the number of the fruits exceeding 90 g in weight and the total weight of these fruits, the differences were not significant when comparing the cultivar ’Cibere’ grafted onto the rootstock ’Robusto’ to the cultivar ’Cinema’. The number and weight of the fruits were outstanding in both cases. Compared to the grafted plants, both non-grafted cultivars produced smaller numbers of fruits over 90 g in weight. Although the differences were not significant, ’Cibere’ grafted onto ’Snooker’ and ’Citera’ grafted onto ’Snooker’ produced more fruits over 90 g in weight by approx. 20% and almost 90%, respectively. There were no significant differences when comparing the grafted plants to the cultivar ’Cinema’, and only the non-grafted ’Citera’ and the resistant cultivar No. 2 produced smaller numbers of fruits of the aforementioned category than the grafted plants and the cultivar ’Cinema’ did. This result shows that the size of the fruits remained small in the case of the resistant cultivar No. 2 in spite of the fact that it produced many fruits.

As ’Cinema’ produced more or statistically equal yield compared to the grafted plants, it was thereafter compared with other high-yielding cultivars and grafted plants to clarify its yielding properties and the adverse effects of the root-knot nematode species on the yield as much as possible.

In 2009, there was no significant difference, in the case of either cultivar, in respect of the total weight of fruits per plant when comparing the grafted plants to the non-grafted ones of the same cultivar to each other. Although the cultivar ’Cibere’ grafted onto the rootstock ’Snooker’ produced more yield by 18%, and the cultivar ’Verecke’grafted onto the same rootstock yielded less by 22%, the difference did not prove to be significant in either case. The cultivar ’Verecke’ produced the most yield if it was non-grafted, and the difference was significant when comparing it to the non-grafted cultivars ’Cibere ’ and ’Century’, but the difference was not significant when comparing it to the non-grafted cultivars ’Cinema’, ’Creta’ and ’Kurca’. The non-grafted cultivars

’Verecke’, ’Kurca’ and ’Creta’ produced more yield compared to the grafted plants, but the differences were not significant. The cultivar ’Century’ grafted onto either of the two rootstocks produced more yield by 10%, but the differences were not significant.

In 2010, there was no significant difference, in the case of either cultivar, in respect of the total weight of fruits when comparing the grafted plants to the non-grafted ones of the same cultivar to each other. Probably due to the cooler summer, the mean yield increased considerably in this year. In 2010, the plants were transplanted about three weeks later than they were in the previous year, therefore, the shorter growing period might also contribute to the advantageous effects of the rootstocks on the scions to be expressed only to a lesser extent. The ability of the rootstocks of taking up water and nutrients more effectively due to their more developed roots could not be proved.

In 2009, concerning the number of fruits per plant, the cultivar ’Verecke’ produced the most fruits, however, the differences were not significant either in the case of cultivars or rootstock-scion combinations. Only the non-grafted cultivar ’Cibere’ produced a significantly smaller number of fruits than some of the cultivars and combinations did. Nevertheless, if the cultivar ’Cibere’ was grafted onto the rootstock ’Snooker’, it produced more fruits by almost 20% compared to the non-grafted plant. The cultivar ’Century’ grafted onto the rootstock ’Snooker’ produced more fruits by approx. 10%, but the difference was not significant. In 2010, there was no significant difference, in the case of either cultivar, in respect of the number of fruits when comparing the grafted plants to the non-grafted ones of the same cultivar to each other.

In 2009, concerning the weight of the fruits exceeding 90 g in weight, the cultivar ’Verecke’ grafted onto the rootstock ’Snooker’ yielded significantly less compared to the non-grafted plant. There was no significant difference, in the case of either of the further cultivars tested, in the aforementioned respect when comparing the grafted plants to the non-grafted ones of the same cultivar to each other, although, similarly to the total weight of fruits, the grafted plants yielded less than the non-grafted ones did. The smallest number of fruits over 90 g in weight was produced by the cultivar ’Century’. In 2010, the cultivar ’Creta’ produced the highest yield of fruits over 90 g, while the root-knot nematode resistant cultivar ’Cinema’ came second. In this respect, the cultivar ’Kurca’ yielded significantly less compared to the cultivar ’Creta’. In the same year, the cultivar ’Kurca’ grafted onto the rootstock ’Robusto’ produced significantly more fruits over 90 g by 20%, but the difference was not significant. The amount of fruits of extra size produced was almost the same when comparing either the cultivar ’Cibere’ or ’Verecke’ to the grafted combination.

b) The effects of using pepper cultivars resistant to *Meloidogyne incognita* and those of grafting onto resistant rootstocks on the occurrence of blossom-end rot in pepper

During the experiments on grafted peppers, the number of fruits showing the symptoms of blossom-end rot (BER) was recorded. In 2009, the number of fruits with the symptoms of calcium deficiency in the case of the cultivar 'Cinema' was smaller than it was in any of the other non-grafted susceptible cultivars, however, this difference was significant only in the cases of the cultivars 'Cibere' and 'Kurca'. In the cases of the grafted peppers, in the same year, the number of fruits with BER was smaller when the plants grafted onto either rootstock were compared to the non-grafted ones of the same cultivar. In 2010, the results confirmed the finding that the number of fruits with BER in the cases of both combinations was the half of that counted in the non-grafted susceptible plants. When grafted onto the rootstock 'Robusto', the cultivars 'Kurca' and 'Century' produced significantly smaller number of fruits showing BER compared to the non-grafted plants.

With the exception of the cultivar 'Creta', all the grafted plants had smaller numbers of fruits showing the symptoms of BER compared to the non-grafted ones in 2010. In several cases, the number of fruits with BER in the cases of the grafted plants was smaller than the half of that counted in the non-grafted plants of the same cultivar. However, the difference was found to be significant only when the cultivar 'Verecke' was compared to the cultivar 'Verecke' grafted onto the rootstock 'Robusto'. The resistant cultivar 'Cinema' produced a significantly smaller number of fruits with BER than three other non-grafted cultivars did in this year. Among the non-grafted cultivars susceptible to the root-knot nematode species, the smallest number of fruits with BER was found in the cultivar 'Creta', probably because of the lower susceptibility of this cultivar to the pest compared to that of the other ones.

Due to their resistance to the root-knot nematode species and their stress tolerance (saline soils, temperature fluctuations), too, the rootstocks probably contribute to the better nutrient supply of the plants, and thus, the rate of fruits showing BER is smaller in the cases of the grafted plants compared to the non-grafted ones. In the case of the cultivar 'Cinema', the resistance to the nematode species and other favourable properties of the cultivar may increase the number of healthy and marketable fruits without the symptoms of calcium deficiency.

IV. NEW SCIENTIFIC RESULTS

1. The resistance of 80 pepper cultivars and breeding lines to *Meloidogyne incognita* has been established to help the breeders' work so that root-knot nematode resistant cultivars may become available to the Hungarian growers as soon as possible.

2. The resistance of the commercially available rootstocks for melon and that of some other potential rootstocks belonging to the family Cucurbitaceae to *Meloidogyne incognita* has been established.

3. Based on the results of the studies on the members of the family Cucurbitaceae, it has been established that because of the considerable differences concerning the weight of roots in the cases of the different species, the assessment of resistance to the root-knot nematode species might be carried out more precisely by taking the number of galls per a unit of root weight into consideration instead of counting the total number of galls per root.

4. The susceptibility of *Echinocystis lobata* (wild cucumber), a potential rootstock for melon, to *Meloidogyne incognita* has been established. According to my results, it was infested to a similarly slight extent to *S. angulatus*, which is used as a rootstock for cucumber for the control of *Meloidogyne incognita*. Therefore, investigation on its compatibility with scions is proposed to carry out.

5. It has been established that the growing of the cultivar 'Cinema', the first commercially available root-knot nematode resistant pepper cultivar in Hungary, may give the chance of controlling root-knot nematodes effectively and environmentally friendly in cold forcing.

6. It has been found that the advantages of using grafted pepper plants whose rootstock is resistant to *M. incognita* for the control of the nematode species might not be reflected in the case of growing in cold forcing-houses for a short period.

7. It has been found that the environmentally safe control of root-knot nematodes which is based on the choice of cultivars plays a role in the the control of blossom-end rot, which is the most significant abiotic disease of pepper. By using the grafted plants or the resistant cultivar, the number of fruits showing the symptoms of calcium deficiency might be reduced effectively.

V. DISCUSSION

Several tested breeding lines belonging to the species *Capsicum annuum* showed resistance to the southern root-knot nematode. The results of my studies on the cultivars that were considered to be promising for resistance breeding to control *M. incognita* have been given to breeders to help their work. Due to the intensive breeding work, the first root-knot nematode resistant cultivar belonging to the sweet, white-fleshed group of pepper cultivars has become available in Hungary since the beginning of our studies. The development of further pepper cultivars of other groups resistant to *Meloidogyne* species that require high temperature for their development might be expected.

The results of the studies in which seeds of the tested pepper plants were sown in soil infested with *Meloidogyne incognita* call our attention to the adverse effects of using infested medium for the growing of seedlings. The condition of the pepper plants that became infested as seedlings is much worse than that of the originally healthy plants that became infested only at the time of transplanting. That is why the use of uninfested transplants has to be emphasized.

According to the results of the assessments of resistance of the members of the family Cucurbitaceae to *M. incognita*, by taking the number of galls per a unit of root weight into consideration, the melon cultivar that served as a control proved to be highly susceptible to the southern root-knot nematode. On the basis of my results, the use of the interspecific cultivars as rootstocks, because of their susceptibility, can not be recommended if the soil is infested with root-knot nematodes. This finding has also been confirmed by the experience of growers.

I found neither *Sycios angulatus* nor *Cucumis metuliferus* to be absolutely resistant to *M. incognita*. Compared to the melon cultivar used as a control, the extent of damage was smaller in the cases of the former plant species; however, the difference was not significant in either case. Nevertheless, their testing as rootstocks in melon production is proposed.

The *Lagenaria* cultivars might be able to tolerate the damage caused by nematodes due to their large root weight.

It has been found that because of the root weight loss that results in the reduction of the total number of galls, and also because of the considerable root weight in the cases of the *Lagenaria* rootstock cultivars, the assessment of resistance to the root-knot nematode species might be carried out more precisely by taking the number of galls per a unit of root weight into consideration instead of counting the total number of galls per root.

No literature has been found on the susceptibility of *Echinocystis lobata* (wild cucumber). According to my results, it was infested to a similarly slight extent to *S. angulatus*, which is used as a rootstock for cucumber for the control

of *Meloidogyne incognita*. Therefore, investigation on its compatibility with scions is proposed to carry out.

In respect of both the weight and number of fruits, the cultivar ’Cinema’, which is resistant to *M. incognita*, significantly exceeded the non-grafted susceptible cultivars in 2008. In 2009 and 2010, it produced high yield again, but the difference in yield was not significant in comparison to several non-grafted susceptible cultivars. During the years of the experiments, the extent of damage caused by the root-knot nematode species was decreasing, which was undesirable in respect of the aim of the studies. The reason for this might be the negative effect of the use of resistant cultivars on the root-knot nematode population, which was proved by Thies et al. (2004) and Thies et al. (2005). As the damage considerably decreased in the latter years compared to that observed in 2008, the differences also decreased in comparison of the resistant and the susceptible cultivars. This latter phenomenon might also be affected by the cooler and wetter summer of 2010. On the basis of my results, in the cases of unheated greenhouses, the use of resistant pepper cultivars is primarily recommended for growers to control *Meloidogyne incognita* in Hungary. The advantages of using grafted pepper plants whose rootstock is resistant to *M. incognita* for the control of the nematode species might not be reflected enough in the case of growing in cold forcing-houses for a short period. A better return on the investment in using grafted plants might be expected in the case of growing in heated greenhouses.

The results show that the alternative and environmentally friendly, chemical-free nematode control methods (grafting onto resistant rootstocks or growing resistant cultivars) may contribute to the control of blossom-end rot (BER), the occurrence of which is partly associated with the damage caused by the pest. Due to their resistance to the root-knot nematode species and their stress tolerance (saline soils, temperature fluctuations), too, the rootstocks probably contribute to the better nutrient supply of the plants, and thus, the rate of fruits showing BER is smaller in the cases of the grafted plants compared to the non-grafted ones. Some cultivars, such as ’Creta’, seem to be less likely to produce fruits showing the symptoms of BER. Although approximately 35% less calcium was applied per square meter in 2010, the number of fruits with BER did not increase. In the case of the cultivar ’Cinema’, the resistance to the nematode species and other favourable properties of the cultivar may increase the number of healthy and marketable fruits without the symptoms of calcium deficiency.

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