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Effect of some root leachates and dry extracts of Brassicaceae plants on potato cyst nematode populations

Katarzyna Franke, Grzegorz Gryń, Mateusz Maciej Nowakowski, Mirosław Nowakowski and Barbara Skibowska

Plant Breeding and Acclimatization Institute – National Research Institute, Poland
e-mail: k.franke@ihar.bydgoszcz.pl

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Summary. The potential effect of *Brassica* plants for control of the potato cyst nematode, *Globodera rostochiensis*, was assessed in a laboratory experiment and pot test. Second-stage juveniles (J2) hatching in the absence of a host plant could reduce levels of *G. rostochiensis* populations in the field. The effect of several Brassicaceae plant extracts and root leachates on hatching of *G. rostochiensis* invasive J2 was tested in laboratory conditions. *Raphanus sativus*, *Sinapis alba* and *Lepidium sativum* were tested. The controls consisted of root leachates obtained from *Solanum lycopersicum* and *S. tuberosum*, plus leachate from pots without plants and tap water. Host plant leachates stimulated the greatest hatch (over 95%). Among the non-host plants studied, the highest hatch stimulation was with root leachate from *L. sativum* (53.3%). Large variation in hatching was found for cultivars of *R. sativus* (20.8% and 33.6%). In the pot experiment, the objective was to determine the inhibitory effect of dry matter extracts from six plants, added to the soil, on the development of the *G. rostochiensis* population in the cultivation of a susceptible potato cultivar 'Desiree'. Two *R. sativus* cultivars ('Colonel' and 'Romesa'), two *S. alba* cultivars ('Bardena' and 'Concerta'), *L. sativum* and *Nasturtium officinale* were chosen for the tests. No extract was used for the negative control, and for the positive control, Vydate® 10G nematicide was added. Among the extracts studied, *L. sativum* extract limited development of the nematode population (75%) closest to the chemical treatment.

Keywords: biocontrol, *Globodera rostochiensis*, nematode control, nematode hatching, root leachate.

Globodera rostochiensis (Wollenweber) Behrens, the potato cyst nematode (PCN) belonging to the family *Heteroderidae*, is a serious threat to potato cultivation worldwide. The species originates from the Andean region and has spread to almost all parts of the world where potatoes are grown. It is classified as a quarantine organism in many countries in Northern America, Africa, Europe and Asia, including USA, Canada, Mexico, Norway, Belarus and Poland. Official procedures and methods regarding control of this pest are not uniform or equally restrictive in all European Union countries. There were differences in principles and practice of sampling for PCN detection, differences in actions taken when PCN was detected, and management and regulatory control strategies in cultivation of potatoes differed (Karnkowski, 2015).

Problems in eradicating the nematode populations result from the uneven distribution of cysts in the field, which pose a threat to potato cultivation for several years. Among the effective methods of limiting PCN numbers, apart from quarantine treatments (Kornobis, 1982; Malec, 1985), the cultivation of resistant potato cultivars, use of sprouted potatoes (effectiveness of up to 90%) and cultivation of trap plants (Rychlik *et al.*, 2005) can be utilised. In 2018, in the Polish National List of Potato Varieties there were just a few genotypes included, which are susceptible to *G. rostochiensis* pathotype Ro1, and a small pool resistant to other nematode pathotypes. The import of foreign potato cultivars in Poland, as well as simplified rotation patterns, increases the risk of introduction and reproduction of other pathotypes. Przetakiewicz (2013) reported the first outbreaks of potato cyst nematode Ro5 pathotype in Poland.

The cultivation of non-host plants should be considered as an element of integrated potato

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protection. They can decrease the potato cyst population by up to 50% (Kornobis, 1982). The cultivation of catch crops, introduced to the crop rotation, contributes to reducing the nematode population in the soil and may be of great importance. In the available literature, researchers confirm the nematicidal activity of species belonging to family Brassicaceae (Lord *et al.*, 2011; Fatemy & Sepideh, 2016). The mechanism responsible for these properties was described by Valdes *et al.* (2011), Brolsma *et al.* (2014) and Fourie *et al.* (2016). Due to the presence of glucosinolates, whose hydrolytic degradation products show toxicity against other organisms, cabbage plants may contribute to limiting the population size of *G. rostochiensis* in the soil.

The aim of this study was to assess the impact of selected Brassicaceae species on PCN in two ways. Firstly, the hatching stimulation effect of root diffusate in laboratory conditions was verified and, secondly, the influence of dry matter on a population of PCN during cultivation of a susceptible potato variety was observed.

MATERIAL AND METHODS

The hatching of *G. rostochiensis* invasive J2 from cysts under the influence of selected plants from the Brassicaceae family was evaluated in two studies: I. In laboratory conditions, the effect of root leachate on hatching was analysed; II. A pot experiment assessed the negative effect of dry plant extracts on the development of the nematode population.

I. Hatching stimulation by root leachate. In the study, 96-well plates (Nest Biotechnology Co., Ltd), cyst nematodes of *G. rostochiensis* pathotype Ro1 and root leachates of species of Brassicaceae plants, controls of potato and tomato root leachates and tap water were used.

***Globodera rostochiensis* population.** The cysts of *G. rostochiensis* Ro1 were obtained from a single generation of the population maintained in the PBAI-NRI laboratory, Division in Bydgoszcz. A decontaminated substrate composed of 25.8% high peat, 61.4% transitional peat, 12.8% sand and N-NO_3 – 138 mg l^{-1}, P – 63 mg l^{-1}, K – 180 mg l^{-1}, Mg – 94 mg l^{-1}, Ca – 1817 mg l^{-1}, pH in H_2O – 6.2 content; mineral fertilisers were added as recommended for potato cultivation. Potatoes ‘Desiree’ were planted in 1 l pots. Potato cyst nematodes Ro1, packed in nylon bags (2 × 2 cm) with a pore diameter of 0.22 mm, were added. Pouches made of perforated material allowed the J2 to move freely into the soil. A nematode inoculum containing 10 ± 5 live J2 and eggs for each ml of soil substrate was used.

After 15 weeks, the potato plants were dried to complete the test. Plant residues, tubers and bags were removed from the pots. The substrate was dried and sieved to obtain the appropriate granulometric fraction, *i.e.* 0.25-1.25 mm. The cysts were extracted using a simplified bottle method (Marks & Brodie, 1998) and stored in plastic containers at 4°C for 4 weeks. Before use, the cysts were soaked in water for 24 h.

Root leachate. Root leachate is an aqueous solution containing root secretions obtained by transferring the content of pots used for cultivation of selected plants. These substances, produced by host plants, stimulate the hatching of J2 from cysts. The root leachates were prepared from separate pots containing two oilseed radish (*Raphanus sativus* L.) cultivars: ‘Colonel’ and ‘Romesa’, white mustard (*Sinapis alba* L.) ‘Bardena’, cress (*Lepidium sativum* L.), tomato (*Solanum lycopersicium* L) ‘Moneymaker’, and potato (*Solanum tuberosum* L) ‘Miłek’. ‘Miłek’ is an early cultivar that grows well in pots and is resistant to PCN Ro1. As a control, soil leachate was used, obtained from a pot filled with soil without any plants. Plants were sown in 12 l pots, grown under controlled temperature: 21°C – 12 h / 18°C – 12 h, and systematically watered. All leachates were collected 6 weeks after sowing plants, according to the method described by Wilski (1973) and Twomey *et al.* (1995), then pre-filtered through a filter paper and stored at 4°C.

Experimental conditions. Before use, root leachates were filtered using syringe filters (Sartorius, cellulose acetate, 28 mm, 0.2 μm). Exactly 200 μl of the analysed leachate and one cyst were placed in each well of the titration plate. All leachates and control were tested on 120 cysts. Every cyst contains 120-150 juveniles and was tested separately. One sample consisted of one cyst. The titration plates were closed, covered with Parafilm® M, wrapped with aluminum foil and stored under constant temperature 21°C – 12 h / 18°C – 12 h. Hatching was evaluated after 1, 2, 3 (data not presented) and 4 weeks, to be sure that hatching is completed, then the tested leachate was removed and each cyst was covered with tomato leachate. After 10 days, the numbers of live eggs and juveniles in the cysts were evaluated. Observations were done using an inverted microscope (Olympus CK2, at ×25). In order to facilitate the analysis the six-category scale was used for evaluation: 0 – no hatching of J2, 1 (1 – 5 J2 in a well of the titration plate), 2 (6 – 20), 3 (21 – 50), 4 (51 – 100) and 5 (> 100); for analysis hatching after the 4th week and after tomato leachate results were used.

II. Influence of dry matter plant extracts on the *G. rostochiensis* population size in the susceptible potato cultivation. In the pot experiment, dry matter extracts of cabbage plants, *G. rostochiensis* pathotype Ro1, and 'Desiree', a susceptible potato cultivar, were used.

***Globodera rostochiensis* population.** Cysts of *G. rostochiensis* pathotype Ro1 were obtained by reproduction in controlled conditions, as described above for the laboratory experiment.

The evaluation of live eggs and J2 number was made on three cyst samples, 100 cysts in each. Cysts from a single generation were selected and placed in nylon bags with a mesh diameter of 0.22 mm. The nematode inoculum contained 2,500 live eggs and J2 in each pouch.

Plant material. Plant material was obtained from the cultivation of oilseed radish (*R. sativus*) 'Colonel' and 'Romesa', white mustard (*S. alba*) 'Bardena' and 'Concerta', watercress (*Nasturtium officinale*) and cress (*L. sativum*). Plants were sown in pots filled with growing medium in controlled conditions (21°C – 12 h / 18°C – 12 h) and watered regularly. The 8 week-old plants were cut, dried, and milled in a laboratory mill. The prepared dry matter was stored in sealed plastic containers for about 2 weeks.

Experimental conditions. The experiment was performed in controlled conditions of the phytotron. For each 3 l pot, 5 g of the dry extracts were used, in six replicates for each combination; 24 t ha^{-1} of fresh matter (calculated for white mustard fresh matter); soil substrate with the following characteristics was used: soil reaction acidic, N-NO_3 – 24 mg $(dm^3)^{-1}$, P – 52 mg $(dm^3)^{-1}$, K – 184 mg $(dm^3)^{-1}$, Na – 93 mg $(dm^3)^{-1}$, Ca – 1450 mg $(dm^3)^{-1}$, Mg – 50 mg $(dm^3)^{-1}$). For the negative control, potatoes were planted without the addition of extracts. An additional combination included pots with chemical treatment in the form of Vydate® 10G, at the dose recommended by the manufacturer (10 kg ha^{-1} adjusted in proportion to the pot surface), applied and mixed to the top layer of the substrate on the planting day.

At the edge of each pot, one seed potato was planted. A nylon bag containing cyst nematodes was placed on the opposite side. Three weeks after planting the potatoes, the herbal extracts were added by gently mixing with the top layer of the substrate. Throughout the growing period, the potatoes were watered so that the water permeated through the extracts into the soil. After 3 months, the plants were removed and a 100 ml soil sample was taken to determine the size of the potato cyst nematode population. The number of cysts, eggs and juveniles were counted. The obtained data were used to assess the impact of the tested extracts on the development of the nematode population. The number of eggs and J2 that remained in the cysts in the pouches were also counted (using an inverted microscope). The results were statistically analysed using one-way ANOVA (STATISTICA® 9.0), and the differences between means were compared using the LSD test at the significance level $P = 0.05$. The evaluation of the investigated extracts effectiveness was performed according to the Henderson-Tilton formula.

RESULTS

I. Effect of root leachate on hatch stimulation. Microscopic observations revealed a varied response of J2 of *G. rostochiensis* to root leachates of the plants studied. Hatching occurred already in the first week, while being most intense in the third week. After applying the tomato root leachate, an increase in hatching was recorded. The number of J2 that left the cysts was counted and grouped according to the scale. The results are summarised in Figure 1, with columns representing the percentage hatching from cysts by week 4 in response to the tested leachate. The curve in the upper part of Figure 1 shows the percentage of live juveniles in cysts, verified after exposure to tomato root leachate.

The greatest hatch was from cysts in potato root leachate. After exposing cysts to leachates from tomato and potato, the total hatch was 95.0% and 96.7%, respectively. In the group of Brassicaceae plants, the highest hatch (53.3%) was reported for the cress extract treatment. A 50% rate was found with leachate from pots without plants. The lowest hatch was found with root leachates of oilseed radish 'Romesa'. Significant differences ($P < 0.05$) (of 13% hatch) between the two oilseed radish cultivars were reported.

The percentage hatch using the six-categories scale was also analysed. Mass hatching was found only with the host plants. It reached a level of the fourth (51-100 J2) and fifth (> 100 J2) category. Hatching at the level higher than 50 J2 was found in over 70% of cysts tested. Among the analysed root leachates of cabbage plants, the third category of hatching (21-50 J2) was reported in cress at the highest level 9.2%. Root leachates from oilseed radish 'Romesa' had the lowest impact on hatching.

Secondary treatment of cysts with tomato leachate resulted in increased hatch of J2. The percentage of cysts with live J2 was estimated at over 85% for all plants tested.

Table 1. Rating size of starting population of *Globodera rostochiensis* before treatment with dry matter from six Brassicas. Controls consisted of no treatment (negative control) and treatment with the nematicide Vydate® 10G (positive control).

Treatment	Mean number of J2 and eggs in cysts from the bags* [pcs.]**
Untreated	$11.3^{c} \pm 12.1$
Nematicide	$29.18^{ab} \pm 6.7$
White mustard ‘Bardena’	$17.6^{bc} \pm 6.0$
White mustard ‘Concerta’	$37.5^{a} \pm 7.5$
Cress	$20.3^{bc} \pm 4.8$
Watercress	n.t.***
Oilseed radish ‘Colonel’	$26.0^{ab} \pm 2.2$
Oilseed radish ‘Romesa’	$30.0^{ab} \pm 14.5$

$^{a,\ b,\ c}$ mean values indicated by the same letters are not statically significant at the 0.05 level by LSD test.

* Mean ± SE (standard error) of six replications.

** pcs. = pieces.

*** – not tested.

J2 = second-stage juveniles.

II. Influence of dry matter extracts on the development of *G. rostochiensis* population in the cultivation of a susceptible potato cultivar. Potato plants were observed during vegetation. There were no differences in the plants between combinations treated with extracts or without treatment. During the flowering period, the presence of cysts on the outer root sheath was found in each combination.

The characteristics of the starting population are shown in Table 1. Not all cysts were empty after completing the study. The lowest number of eggs and J2 remained in the cysts in pots with no treatment, while the highest was in the combination with white mustard ‘Concerta’.

After completion of the experiment, the results were statistically analysed and summarised in Table 2. The highest number of cysts was produced in control pots and with the addition of watercress extract. The lowest value was found in the combination with chemical protection, which was significantly ($P < 0.05$) different from the other treatments. As for the plants studied, significant differences in mean number of cysts in 100 ml of soil were not reported. The influence most similar to the Vydate® 10G control was found in the treatment with the addition of cress extract.

The mean numbers of eggs and J2 of the new generation of *G. rostochiensis* varied with the treatments. The highest number of J2 was found in the control, without treatment, while the lowest was in the combination with chemical treatment. Results for the cress and both white mustard and oilseed radish varieties extracts differed significantly from the control. Treatment with watercress resulted in the lowest impact on reducing mean number of eggs and J2 in cysts.

The effectiveness of limiting the cyst population densities in the range of 20-50%, which according to Kornobis (1982) indicates the nematicidal activity of non-host plants, was reported for the combination with the addition of watercress and white mustard ‘Bardena’. An even higher effectiveness was found

Table 2. Assessment of newly formed populations of *Globodera rostochiensis* in pots with potato ‘Desiree’ after treatment with dry matter from six Brassicas. Controls consisted of no treatment (negative control) and treatment with the nematicide Vydate® 10G (positive control).

Treatment	Mean number of cysts in 100 ml of soil [pcs.]*	Mean number of eggs and J2 in cysts in 100 ml samples of soil [pcs.]*	Percentage of effectiveness [%]**
Untreated	$32.8^{a} \pm 7.8$	$2774.0^{a} \pm 446.3$	–
Nematicide	$3.0^{c} \pm 0.7$	$78.0^{d} \pm 41.5$	97.2
White mustard ‘Bardena’	$18.4^{b} \pm 9.4$	$1368.0^{bc} \pm 234.7$	50.7
White mustard ‘Concerta’	$16.6^{b} \pm 8.2$	$1262.0^{bc} \pm 217.6$	68.0
Cress	$11.6^{bc} \pm 4.8$	$678.0^{cd} \pm 393.5$	75.6
Watercress	$21.3^{ab} \pm 6.3$	$1873.0^{ab} \pm 716.5$	32.5
Oilseed radish ‘Colonel’	$16.6^{b} \pm 2.4$	$1250.0^{bc} \pm 301.1$	55.0
Oilseed radish ‘Romesa’	$14.0^{b} \pm 2.3$	$984.0^{bcd} \pm 500.6$	72.3

$^{a,\ b,\ c,\ d}$ mean values indicated by the same letters are not statically significant at the 0.05 level by LSD test.

* Average ± SEM (standard error mean) of six replications.

** Percentage of effectiveness according Henderson-Tilton formula.

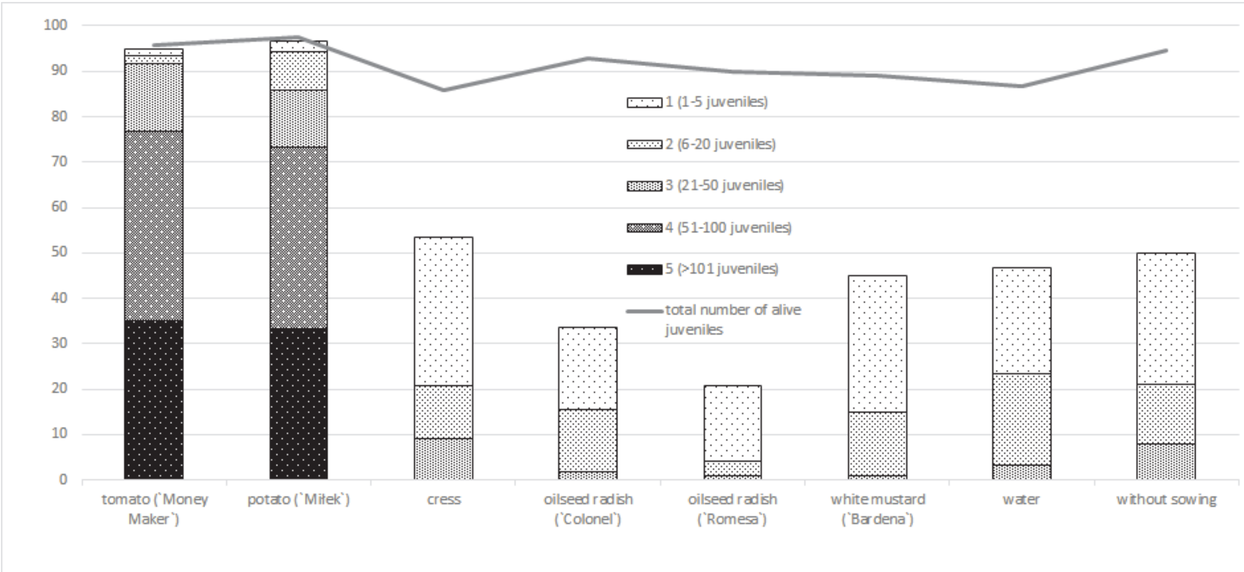

Fig. 1. Mean percentage hatch of second-stage juveniles (J2) from cysts of *Globodera rostochiensis* after treatment with leachates from six Brassicas. The hatch category scale: 1 (1-5 J2 in a well of the titration plate), 2 (6-20 J2), 3 (21-50), 4 (51-100 J2), 5 (> 100 J2).

for cress (75.5%), oilseed radish (‘Romesa’: 72.3%, ‘Colonel’: 54.9%) and white mustard ‘Concerta’ (67.9%) extracts.

DISCUSSION

To increase the efficiency and accelerate the speed of elimination of potato cyst nematode populations, it is important to include plant species in the crop rotation that will at least slightly reduce the size of the pest’s population. Introducing intercrop plants as a phytosanitary factor is becoming increasingly popular. Oilseed radish and white mustard cultivation is frequently reported. However, the effect of white mustard on the population of potato cyst nematode in the soil is cultivar-specific (Pastuszewska *et al.*, 2013). The selection of desirable traits takes place at the genotype and variety level (Nowakowski *et al.*, 2014). Cultivar-specificity was also found in the present study.

In the first experiment, the greatest ability to stimulate hatching of *G. rostochiensis* invasive J2 was demonstrated by host plants, mainly potato (up to 95%). In practice, resistant cultivars, whose presence in the field causes a massive hatching of J2 from cysts, are of great importance for limiting the development of the pest’s population. A necrotic reaction on the root at the site of nematodes penetration stops the development (Wilski, 1973). The cultivation of a resistant potato cultivar results in clearance the entire field after only a few years of monoculture (Zawiślak *et al.*, 1989). Byrne *et al.* (1998) claim that roots from non-host plants contain other substances that may have a similar effect on J2 hatching.

Valdes *et al.* (2012) found that cysts infused for 2 weeks with root leachate produced from white mustard, rape and radish did not lose the ability to hatch under the influence of tomato leachate. Similar results were obtained in the present study, despite the treatment of cysts with the analysed root leachate for 4 weeks. The highest hatching rate was reported in the third week of observation. The evaluation performed after transfer of the cysts from the test leachate to the control tomato leachate showed increase of hatching, which indicates that the tested leachates did not change the number of live J2 in cysts, did not inhibit hatching from cysts, and did not contain any hatch-stimulating compounds. Malec and Bogaczyk (1978) studied the influence of several crops and weeds on the hatching of J2 from cysts in a pot experiment. They found little significance of fodder beet, tobacco and white lupine cultivation. In the present study, there was a slight hatching effect of leachate from roots of oilseed radish ‘Romesa’ (20.8%) and ‘Colonel’ (33.6%).

In the second part, the nematicide effect of soil amendments was tested. Significant reduction of *G. rostochiensis* population size was demonstrated by López-Lima *et al.* (2013) in a field experiment.

Selected combinations of the studied rotation, *e.g.* with the addition of peas and beans, resulted in a reduction of the nematode population by up to 89%. Trifonova (2003) assessed the effect of 'green fertilisers' produced from barley plants, black mustard, field pea and marigold on the invasive J2. The best effects were reported with mustard, which caused a reduction of the potato cyst nematode population by 79% as compared with the control. An alternative to green manures is incorporation of dry milled matter to potato rotation. For effective management of potato cyst nematodes it is important to combine different methods.

Incorporating plant residues into the potato cyst nematode infested soil could be an alternative to chemical protection. This manuscript presented an introductory experiment. Plants used for preparing extracts were cut in the flowering growth phase as recommended by Fourie *et al.* (2016). In contrast to many studies on green manure or 'green fertilising' incorporation to the soil (*e.g.* Valdes *et al.*, 2012; Ngala *et al.*, 2014), the aim of the present study was to check the potential of dry matter controlling *G. rostochienis* population. Dry matter for potato cyst nematode control was as used by Aires *et al.* (2009) and Pourmehdi Zanbouri & Fatemy (2014). *Brassica* plants have been tested for control in other experiments. For example, dry leaf tissues amendment caused mortality of *Pratylenchus neglectus* (Potter *et al.*, 1998), and some dried *Brassica* plants pellets reduced the inoculum density of *Pythium irrerulare* and some *Meloidogyne* spp. (Oliviera *et al.*, 2011).

It is known that even post harvested drying causes significant losses of glucosinolates activity (Yábar *et al.*, 2011). For laboratory conditions, it is an easy and quick way to prepare and store this type of extracts. There are some advantages in using dried plant material; it takes up to 120 days to produce fresh green manure, which is not always commercially feasible. Dry material could be produced in fallow fields, then harvested and stored for future needs. It makes for easy storage and more flexibility when required for application.

In the study with other cabbage species, Aires *et al.* (2009) found a relation between the level of glucosinolates in plants and the number of newly formed cysts. The most effective response was reported with watercress. In the present study, watercress did not demonstrate better effects than other plant species. Serra *et al.* (2002) found that the reaction of potato cyst nematodes to glucosinolates derived from watercress seeds occurred only in the presence of synthetic myrosinase.

The most effective treatment was reported with cress, which caused the greatest reduction in the nematode population size (75.6%). The assessment of J2 mortality under the influence of water extracts obtained from spearmint, cress and plants from the Brassicaceae family was also performed by Pourmehdi Zanbouri & Fatemy (2014) and Fatemy & Sepideh (2016). According to these authors, mortality reached almost 100% when the extracts were applied to J2.

Results from the present study confirm the influence of cabbage plants on the population of *G. rostochiensis* in the soil. The leachates and extracts produced under controlled conditions had little effect on the population size changes. Noteworthy are the results from the pot experiment, as the conditions are more similar to those soil conditions in the field. It is necessary to analyse also other soil-related factors, such as fungi, bacteria and processes occurring during plant vegetation. Perhaps their presence affects the activity of cabbage plants related to the hatching of invasive juveniles.

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Katarzyna Franke, Grzegorz Gryń, Mateusz Maciej Nowakowski, Mirosław Nowakowski and Barbara Skibowska. Влияние некоторых корневых экссудатов и сухих экстрактов растений Brassicaceae на популяции цистообразующей картофельной нематоды.

Резюме. Потенциальный эффект растений *Brassica* за контролем над цистообразующей картофельной нематодой, *Globodera rostochiensis*, был оценен в лабораторном эксперименте и горшках. Вылупление личинок второй стадии (J2) в отсутствие растения-хозяина может снизить уровень популяции *G. rostochiensis* в полевых условиях. Влияние нескольких растительных экстрактов Brassicaceae и корневых экссудатов на вылупление личинок *G. rostochiensis* было проверено в лабораторных условиях. *Raphanus sativus*, *Sinapis alba* и *Lepidium sativum* были протестированы. Контроли включали корневые экссудаты, полученные из *Solanum lycopersicum* и *S. tuberosum*, а также экссудаты из горшков без растений и водопроводной воды. Экссудаты из растений-хозяев стимулировали наибольшее вылупление (более 95%). Среди изученных растений, не являющихся хозяевами, наибольшая стимуляция вылупления наблюдалась из экссудатов корней *L. sativum* (53.3%). Большие различия в вылуплении были обнаружены у сортов *R. sativus* (20.8% и 33.6%). В эксперименте с горшком целью было определить ингибирующее действие экстрактов сухого вещества из шести растений, добавленных в почву, на развитие популяции *G. rostochiensis* при выращивании восприимчивого сорта картофеля 'Desiree'. Для испытаний были выбраны два сорта *R. sativus* ('Colonel' и 'Romesa'), два сорта *S. alba* ('Bardena' and 'Concerta'), *L. sativum* и *Nasturtium officinale*. Для отрицательного контроля экстракт не использовали, а для положительного контроля добавляли нематицид Vydate® 10G. Среди изученных экстрактов экстракт *L. sativum* ограничил развитие популяции нематод (75%), наиболее близкий к химической обработке.