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The Occurrence, Distribution and Control of Potato Cyst Nematodes in Lebanon

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Abstract

This survey was conducted in Akkar and Bekaa area (Lebanon) to determine the presence and distribution of the potato cyst nematode, *Globodera* spp. A total of 294 soil samples were collected from 32 different regions. The nematode counting in the soil samples from Akkar area revealed that 66 out of 217 samples (30.4%) contained cyst nematodes and 18 (8.3%) of the samples contained eggs. On the other hand, 77 soil samples collected from Bekaa contained cyst nematodes, 69 (89.6%) of which contained eggs. The level of infestation of nematodes was varying from one area to another. The highest level was detected in Akkar area from Klayaat with 2 eggs/1g soil and Tell Abass El Charqi with 1.7 eggs/1g soil, however, the lowest level of infection was detected in soil samples from Tell Biri (0.25 eggs/1g soil) and Abde (0.3 eggs/1g soil). The highest infection in Bekaa area was found in Khiara (5.4 eggs/1g soil) and Riak (5 eggs/1g soil). Eight different treatments using chopped plants were tested in pot experiments to assess their effect on potato cyst nematodes. The results showed that radish (*Raphanus sativus*) and zaatar (*Thymbra spicata*) treatments had total control of PCN whereas marigold (*Tagetes patula*) treatment reduced PCN population from 1.16 eggs/1g soil (*Pi*) to 0.15 eggs/1 g soil (*Pf*). The effect of the essential oils on the hatching of PCN was also examined. All pure essential oils (*Azadirachta indica, Linen, Eucalyptus, Allium sativum* and *Salvia officinalis*) had significant effect ($P<0.05$) on the hatching activity in comparison to the potato root diffusate (PRD). *A. sativum* had the greatest inhibitory effect reducing hatching to less than 0.9% at 5µl/ml followed by *Eucalyptus* which reduced hatching to 4.1% at 10µl/ml. Sage gave almost total inhibition a week after treatment at 10µl/ml.

1. Introduction

Plant-parasitic nematodes are present in virtually all soil types and cause significant economic losses in a wide variety of crops [1]. They attack a wide range of economically important crops of horticultural, agricultural and forest systems. Potato crops can be severely damaged by the two potato cyst nematodes, *Globodera rostochiensis* [2, 3] *and G. pallida* [4]. It has been estimated that in UK the PCN cause yield losses around £50 million (about $82 million) per year [5] or about 9% yield loss of annual national production and the annual loss is increasing. Within the European Union, the annual estimated loss is costing €300 million. The cyst nematodes are known to occur in several

countries, especially in cooler areas of subtropical and tropical regions, as well as temperate regions of the world [6] with significant potential to reduce potato tuber yields up to 80% in heavily infested and uncontrolled fields [7]. Barker and Koenning [8] revealed that yield losses incurred in *G. rostochiensis* susceptible potato averaged 38% compared to 18.3% in resistant potato. Cysts containing up to 500 eggs can survive in soil for over 15 years in the absence of a host crop [9], and over 28 years in dormant stage [10]. In Lebanon, today, there is no estimate of the monetary damage caused by PCN. However, several species of nematodes are also considered as the major limiting factors of potato production. In a survey of plant parasitic nematodes conducted during 1970's [11], PCN was not found in Lebanon. However, small survey conducted in 2000 in Ryakk area reported the discovery of potato cyst nematodes for the fist time [12]. The PCR test confirmed the presence of PCN as *G. rostochensis* species. Neither *G. pallida* species nor pathotypes were detected in the survey [12]. The origin of *G. rostochiensis* is not known, establishing its distribution within the potato growing area, however, is essential. Since the survey was conducted over 15 years ago and on a small scale (Ryhhak), the presence of the *G. pallida* cannot be ruled out. A recent survey carried out in the UK revealed that PCN were present in 64% of sites sampled. Of the populations found, 67% were *G. pallida*, 8% were *G. rostochiensis* and 25% contained both species. The control of cyst nematodes is extremely challenging; it requires relatively large dosages of soil fumigants with toxic and expensive nematicides. Agrochemicals have been playing a major role in meeting yield requirements in world food production. However, concern has arisen out of findings relating to their affects on human health and environment. Some pesticides contain active ingredients that have been shown to act as hormone disruptors, possibly causing loss of fertility, carcinogenesis and mutagenesis. Widespread application to most cash crops means pesticides are present in the ecosystems, aquifers and water systems in the main agricultural areas. In the long-term, this could have repercussions on both the natural environment and human health [13]. Hence, there is a need to develop strategies that reduce their use and employ more environmentally benign methods. The alternatives must be as effective as synthetic nematicides, readily available, affordable, and safer for farmers, consumers, and the environment. Several species of nematodes are also considered the major limiting factors of stone fruits production in Lebanon. Plant extracts which contain volatile compounds, especially essential oils or secondary plant metabolites, have been found to possess antimicrobial, insecticidal and nematicidal activity [14-21]. This study seeks to establish the distribution of the potato cyst nematodes in the main potato growing areas of Lebanon and to determine the effect of plant extracts on the hatching mechanism and plant development in pot experiment.

2. Materials and Methods

2.1. Soil Sampling

Field surveys were conducted between 4 April and 30 May 2015 and 2016 in the Akkar plain and Bekaa plain (Fig 1). A total of 294 soil samples were collected from 26 different areas in Akkar area (Table. 1) and 8 different areas from Bekaa (Table 2). Soil samples were taken using a "cheese-corer" style auger with a half-cylindrical blade at a depth of 20-30cm. The cores sample were collected randomly from each selected field, and were packed in cotton cloth bags to give 1-2 kg of soil. Each sample was transported to the Plant Pathology Laboratory of the Faculty of Agricultural Sciences, Lebanese University.

Figure 1. *Map of Lebanon showing surveyed areas (in colour).*

2.2. Cyst Extraction and Detection

The soils were dried in laboratory at room temperature. Cysts extractions were performed using the techniques described previously [22]. Each soil sample was weighed and divided into sub-samples of 250g. The Fenwick can was filled with water before the sample was introduced and stirred with plastic rod for 2-3 minutes. Cysts were collected in sieves, transferred into muslin and left to dry. The number of cysts present in each extraction was counted under stereoscope. This process was repeated until the survey was finished and the required number of cysts was collected.

2.3. Counting of Eggs

The number of cysts present and their content were estimated by standard methods [22]. The collected cysts were soaked in water for 24 h and gently crushed in water suspension using a pestle to release the egg contents. The released eggs were transferred into 20 ml glass cylinder and 15 ml of water was added. The number of eggs was counted in a 2 ml suspension using stereoscope.

2.4. Root Diffusate Collection

Potato root diffusate (PRD) were collected from 3 weeks old potato plants grown in pots, where the potato plants were left unwatered for 5 days and then irrigated with 1 liter of water that was introduced progressively into the pot at 10 minutes intervals. The leached liquid was collected, filtered and diluted 1: 9 with distilled water (stock solution) and stored at 5℃ until needed.

2.5. Hatching Assay

The effect of essential oils on the hatching of the second-stage juveniles *in vitro*, five different essential oils such as neem, linen, Eucalyptus, garlic and sage were determined. Each test was replicated four times using different concentrations (1 µl/ml, 5 µl/ml and 10 µl/ml) and observed for six weeks. In addition, each batch containing 30 cysts was incubated with 2 ml of stock solution containing the potato root diffusate and different concentration of essential oils. Controls were treated in the same manner without essential oils. At weekly interval, the hatched juveniles were removed and counted and fresh solutions were introduced to each treatment. The experiment was maintained at 23°C±2°C.

2.6. Effect of Plant Bio-products on *Globodera* spp. Population

Infested soils with nematodes were collected from infested fields previously tested for nematodes infestation [20]. Nematodes were identified using morphological characters (CAB International) to the genera level only. Collected soils were thoroughly mixed and divided into several batches. Sub-samples were taken for nematodes extraction using the method described above [22] to determine the population initial *(Pi)* before planting. In this experiment eight different treatments were tested: *Raphanus sativus (*whole fresh plant chopped*,* whole fresh plant soaked, and fresh leaves only), *Azadirachta indica* (dry seeds soaked and dry seeds grinded), *Tagetes patula* (whole fresh plants), *Thymbra spicata* (fresh leaves) *and* chemical nematicide (Vaydate). Control treatment consisted of infested with nematodes soil only. Fresh plant materials were cut into small pieces using pruning shears and 20g of each plant were mixed thoroughly with approximately 1 kg of infested soil and then placed in 1.5 kg plastic pot. Twenty ml of extraction solutions of neem and radish were watered into each pot and 1ml Vaydate mixed with 19 ml water were used. All the solutions were added at 2 weeks intervals. Each pot was planted with a single potato seed. Each treatment was replicated 5 times. Pots were placed in randomized plot design. After two months of potato growth, the final nematode density was determined using Fenwick can technique [22]. The number of eggs /1 g of soil, index and plant parameters (root weight and total fresh plant weight) were recorded.

2.7. Statistical Analysis

Sigma Stat 3.5 program was used for statistical analysis and calculation of statistical parameters (One Way ANNOVA, Tukey test mean and standard deviation was used)

3. Results

3.1. Soil Sampling

The level of distribution (infestation) of PCN in the collected soil samples from different areas in Akkar is presented in Table 1. The counts of nematodes in the soil samples revealed that 66 out of 217 samples tested (30.4%) contained cysts nematodes, but only 18 (26.8%) of the cyst contained eggs. The level of infestation was varying from one area to another. The highest level was detected in Klayaat (2.0 eggs/1g soil) and Tell Abass El Charqi (1.7 eggs/1g soil), however, the lowest level of infection was detected in Tell Biri (0.25 eggs/1g soil) and in Bellanet Hissa (0.3 eggs/1g soil). The results also revealed the presence of other nematode cysts (*Heterodera* spp. not potato host) in some samples which may be confused in appearance with potato cyst nematodes, but were not considered in this survey.

Table 1. *Number of Potato Cyst Nematodes Collected from Different Areas in Akkar Region.*

Area	No. of fields surveyed	No. of samples tested	No. of samples containing cysts	No. of samples free of cysts	Average No. of cysts in 250 g soil	No. of samples containing eggs	No. of eggs/g soil
Tell Abass El Charqi	15	15	8	7	9	3	1.7
Tell Abass El Gharbi	11	11	8	4	10	3	0.5
Jdeideh Joumeh	2	2	1	1	10	0	0
Khouaikhat	13	13	4	9	10	0	0
El Haissa	14	14	2	12	5	0	0
Tell Andy	1	1	1	0	0	0	0
Tell Hmaira	11	11	1	10	3	0	0
Tell Biri	8	8	5	3	5	1	0.25
Aarkai	27	27	8	18	8	4	0.89
Kafer Melki	3	3	0	3	0	0	0
El Sammouniye	2	2	0	2	0	0	0

Area	No. of fields surveyed	No. of samples tested	No. of samples containing cysts	No. of samples free of cysts	Average No. of cysts in 250 g soil	No. of samples containing eggs	No. of eggs/g soil
El Baal	3	3	0	3	0	0	0
Miniara	1	1	0	1	0	0	0
El Cheikh Mohammed	5	5	0	5	0	0	0
Tell Hayat	11	11	1	10	0	0	0
Hokr Ed Dahri	11	11	1	10	0	0	0
Khorbet Jundi	5	5	0	5	0	0	0
Klayaat	12	12	7	5	14	2	2.0
El Cheikh Ayach	10	10	1	9	3	0	0
Qombar	10	10	2	8	7	0	0
Semaqiye	9	9	3	6	7	1	0.35
Abde	12	12	4	8	7	1	0.3
Aarida	10	10	3	7	3	0	0
Bellanet hissa	11	11	6	5	8	3	0.6
Total/Average	217	217	66	151	7.3	18	0.83

In the survey in Bekaa area a total of 77 soil samples were collected. All the collected soil samples (100%) were infected with nematodes. The egg counts revealed that 69 (89.6%) out of 77 soil samples collected contained eggs. The level of infestation of soil sample ranged between 2.3 and 5.5 eggs per g soil (Table 2). The level of infestation with nematodes population varied from one area to another. The highest level was observed (5.5eggs per g soil) in Mansoura, while the lowest infestation (2.3) was detected in soil samples collected from Jeb Janin. The average infestation in the area revealed 3.6 eggs/1g soil.

Table 2. *Number of Potato Cyst Nematodes Collected from Different Areas in Bekaa Region.*

Area	No. of fields surveyed	No. of samples tested	No. of samples containing cysts	No. of samples free of cysts	Average No. of cysts in 250 g soil	No. of samples containing eggs	No. of eggs/g soil
Riak	6	6	6	0	32	6	5.0
Kaa	7	7	7	0	42	6	4.8
Khiara	10	10	10	0	57	9	5.4
Louse	12	12	12	0	35	10	3.3
Mansoura	13	13	12	0	40	10	5.5
Gaze	12	12	12	0	55	12	3.8
Job jannin	7	7	7	0	28	6	2.3
Bar elias	10	10	10	0	45	10	3.6
Total/Average	77	77	77	0	42.0	69	3.6

3.2. Effect of Plant Bio-products on *Globodera* spp. Population

The effect of plant bio-products on the development of potato plants cultivated in infested soil with nematodes are presented in Table 3. The highest plant height was obtained with thyme (zaatar) treatment (96.25 cm), while the lowest was obtained with neem solution treatment (62 cm) in comparison to the control (30 cm). The highest root weight was detected in the case of radish solution treatment (25.5 g), but the root development was reduced (4.17 g) with marigold treatment in comparison to the control plants' roots (4.6 g). The highest total plant fresh weight was obtained with radish treatment (66.1 g), whereas the lowest was obtained in the case of neem seeds treatment (39.8 g) in comparison to the controls (13.4 g). There was no significant difference between the treatments with regards to the plant development, but significant difference was observed between the treatments and the control (P<0.5).

Table 3. *The Effect of Plant Materials on Plant Development Infected with Globodera spp. in Pot Experiment.*

Treatment	Latin name	Plant parts used	Plant height (cm)	Root weight (g)	Total fresh plant weight (g)
Radish	*Raphanus sativus*	whole	75.6±13.63	11.48±5.87	66.1±21.71[a]
Radish solution	*Raphanus sativus*	whole	66.0±10.51	25.5±5.87	45.1±11.35[a]
Radish	*Raphanus sativus*	leaves	64.0±9.65	22.3±6.24	41.0±14.72[a]
Neem solution	*Azadirachta indica*	dry grain	62.0±14.13	23.2±5.48	43.3±23.71[a]
Neem grain grinded	*Azadirachta indica*	dry grain	64.0±6.68	21.2±3.87	39.8±11.71[a]
Marigold	*Tagetes patula*	whole	71.0±24.37	4.17±3.62	46.2±25.66[a]
Thyme	*Thymbra spicata*	whole	96.2±11.08	7.55±0.97	62.7±18.42[a]
Vaydate			76.0±13.73	24±4.17	44.0±16.34[a]
Control			30.0±8.61	4.6±6.82	13.4±12.56[b]

Average ± Std Dev (standard deviation) of 5 replications. Data followed by the same letter in the same column are not significantly different at P<0.05.

The effect of plant bio-product on PCN population in pot experiment are presented in Table 4. Two months after planting, the nematode extractions from the soil showed that the nematodes population decrease form 0.7 eggs/1 g soil to

0 eggs/1g soil. The results showed a significant difference (P<0.5) between treatments and control. Radish, thyme and Vaydate gave a total suppression of PCN population. Marigold treatment gave almost total suppression of PCN (*Pf* = 0.15 eggs/g soil). However, there was no significant difference between treatments.

Table 4. *Effect of Plant Bio-Products on PCN Population.*

Treatment	Number of eggs/g soil		Reproduction index (R=*Pf*/*Pi*)
	Before treatment	After treatment	
Radish solution	5.5	0.0	0.0[a]
Radish leaf	5.5	0.6	0.11[a]
Radish whole	5.5	0.0	0.0[a]
Neem solution	5.5	0.7	0.13[a]
Neem grain	5.5	0.6	0.11[a]
Marigold	5.5	0.15	0.03[a]
Thyme leaves	5.5	0.0	0.0[a]
Vaydate	5.5	0.0	0.0[a]
Control	5.5	3	0.55[b]

The data represent the mean of two experiments (Tukey test)[a,b,c,d] Data followed by the same letter in the same column are not significantly different at P<0.05.

The effect of the essential oils on the hatching of PCN is shown in Table 5. All pure essential oils had significant effect *(P*<0.05) on the hatching activity in comparison to the PRD. The effect of neem was noticed a week after treatment when a total inhibition was detected at all concentrations used. However, when the neem oil was removed the hatching resumed after 3 weeks of incubation. At week 6 the inhibition was permanent; this phenomenon was shown in almost all the treatments except with *A. sativum* when the inhibition was almost unrecoverable. *A. sativum* had the greatest inhibitory effect, reducing hatching to less than 0.9% at 5µl/ml followed by *Eucalyptus*, which reduced hatching to 4.1% at 10µl/ml. Sage gave almost total inhibition in the first week at 10µl/ml. *Eucalyptus* also had an inhibitory effect, but it was not fully expressed until the third week. In the majority of the treatments, the greatest reduction in hatching occurred in the first two weeks.

Table 5. *The Effect of Pure Essential Oils on The Hatching Activities of PCN.*

Exposure Time (week)	PRD	Neem			Linen			Eucalyptus			Garlic			Sage		
		Concentration (µl/ml)			Concentration (µl/ml)			Concentration (µl/ml)			Concentration (µl/ml)			Concentration (µl/ml)		
		1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
1	519	2	3	0	10	8	6	134	16	13	268	5	1	246	156	8
2	202	0	0	0	8	4	7	57	5	3	5	0	26	234	145	0
3	71	0	0	0	2	0	0	0	0	3	1	0	6	23	56	20
4	12	454*	324*	295*	194*	89*	78*	19*	10*	0*	0*	0*	0*	8*	27*	12*
5	0	0	0	0	3	1	0	0	5	0	0	0	0	2	0	0
6	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0
Total	804	456	327	295	209	102	91	211	36	19	274	5	33	513	328	40
Unhatched eggs	115	386	267	285	453	344	405	116	330	450	345	543	604	120	55	436
Hatching%	88.4[a]	54.2[b]	55.1[b]	50.8[b]	31.6[c]	22.8[c]	18.3[c]	64.5[b]	9.1[d]	4.1[d]	44.3[b]	0.9[e]	5.5[d]	81.0[a]	14.4[c]	8.4[d]

PRD, Potato root diffusate. The data represent the mean of two experiments (Tukey test)[a,b,c,d,e] Data followed by the same letter in the same column are not significantly different at P<0.05. * the essential oils were removed to determine if the inhibition of hatching was recoverable

4. Discussion

Nematodes are microscopic worm-like organisms that attack the roots of plants. Damaged roots result in a reduced uptake of nutrients and water. The potato cyst nematodes (PCN) comprise of two species *Globodera pallida* and *G. rostochiensis* and are regarded as major pests of the potato *Solanum tuberosum*. In Lebanon, potato crops are economically important to the local economy as they are cultivated on large areas throughout the country and the economic impact of plant parasitic nematode has not been established. A survey has been done in Rayak area reporting the presence of potato cyst nematode [12]. A small survey (Project GTFS/REM/070/ITA, 2006) [24] concerning the major pests and diseases of potato and table grapes in Lebanon revealed the presence of *Globodera rostochiensis* in small number in Akkar region. In our study, the results confirm the presence of potato cyst nematode in the region. The population level in the surveyed areas varied from one area to another. The results revealed that 31% of Akkar samples contained PCN but only about 27% contained eggs. Whereas in Beqaa area, PCN were detected in all collected samples with higher level of egg content. A survey has been carried out in England and Wales for the presence of potato cyst nematodes (*Globodera rostochiensis*, *G. pallida*). The results showed that population of PCN increased from 42% determined in 1996 to 64% in 2002 indicating significant increase in the distribution of PCN throughout England and Wales [25]. This survey [24] also determined that samples contained 67% of *G. pallida*, 8% of *G. rostochiensis*, and 25% were mixed populations. In our survey the number of eggs in cysts was lesser (5.5 eggs/g soil) in comparison to 10 eggs/g soil in England and Wales [25]. In most cases

population densities were low where 62% of the infestations had a density of less than 10 eggs/g soil. The low population densities in Akaar area suggests that farmers either use resistant cultivars against *G. rostochiensis* or overuse nematicides as control measures to combat the distribution of this species. PCN was detected in small number in ware potato land in Bosnia and Herzegovina [26], indicating that the infestation is relatively recent and that there is a threat of further spread of this quarantine species. Another survey has been carried out in Tunisia to investigate species and distribution of the PCN, *Globodera* spp. The results of the survey showed that 41 of the total 118 investigated fields (37.3%) in the different regions were infested by PCN. Therefore, the discovery of potato cyst nematodes in Lebanon and their distribution in the country should be taken seriously, and strict measures should be taken to prevent its distribution to free PCN areas.

Plant extracts which contain volatile compounds, especially essential oils, have been found to possess antimicrobial, insecticidal and nematicidal activity [14-20] and against nematodes [17-20]. Most recently there have been an increasing trend to use alternative methods of nematode control based on replacing chemical nematicides by natural compounds [18, 19, 27, 28]. In our study, four different plants bio- products were used to determine their effect on the PCN population. The results showed that the development of alternative methods derived from plant or plant bio- products have significant effect on potato plant growth. Radish (*Raphanus sativus)* and thyme (*Thymbra spicata)* treatment gave 100% control of PCN in pot experiment and marigold (*Tagetes patula)* treatment gave almost total control of PCN. The results of recent research show that the biofumigation method is successful in suppressing soil pathogens [29-31]. A study has shown that *Brassica* spp (*e.g*., rapeseed, mustard) because of enzymatic degradation release by-products, have nematode-suppressive effect by interfering with their reproductive cycles [32]. Other mechanisms could also be responsible for the anti-nematodes activity, as it has been shown with *Brassica* leaf manure [27]. There have been reports of *Brassicaceae* extracts being used to suppress G. *rostochiensis*. Two different concentrations of glucosinolates (GSLs) from extracts of broccoli, cauliflower, collards of *B. rapa*, collards of kale, Portuguese cabbage and watercress were used to test their effects on *G. rostochiensis* population [28]. The *Tagetes* effect was found to manifest itself most clearly with *Meloidogyne* sp., *Pratylenchus* sp. and *Tylenchorhynchus dubius* [18, 20, 33]. The nematicidal activity in *vitro* showed that several alpha-poly-thienyls in ppm doses were active against *Globodera rostochiensis*, *Ditylenchus dipsaci* and *Anguina tritici* [34]. Swarup and Sharma [35] reported that *T. erecta* root extracts were lethal or inhibitory to the hatching of *M. javanica* and *M. arenaria.* Belcher and Hussey [36] found that *T. patula* acted as a trap crop to *M. incognita*, but prevented giant cell initiation. Studies with thiophene α-therthienyl a chemical component of *Tagetes erecta* also showed high toxicity against *G. rostochiensis* at concentrations 0.1-0.2 $\mu g\ ml^{-1}$ *in vitro* [33].

Although over 20 major compounds of essential oils were identified from different plants, only carvacrol, linalool, thymol and menthone were the most toxic against the J2 of *M. incognita* at very low concentrations (1mg per liter) [18]. Moreover, several of these oils immobilized juvenile root-knot nematodes and some also reduced hatching of eggs [18]. In this study, all the essential oils gave significant effect on the hatching mechanism of *Globodera sp. A. sativum* totally suppressed the hatching at concentration *5* µl/ml followed by Eucalyptus and sage at 10 µl/ml.

Discussions with some growers during this survey indicated that most of them were unaware of the presence of nematodes and repeated potato production on the same field may contribute to accumulation of high levels of PCN and thus result in yield loss. Further investigations are required to identify the identity of the species and to widen the exploitation of the natural biocidal activity of plant extracts against nematodes as an environmentally benign control measure. In conclusion, the results of this investigation are very important to determine the distribution of PCN in agricultural areas of Lebanon. This knowledge could become essential for the assessment for nematodes management and quarantine measurers.

5. Conclusion

The occurrence and the distribution of potato cyst nematodes in Lebanon have been studied.

The current results revealed that potato cyst nematodes are on the increase in both surveyed areas. All the essential oils tested gave significant effect on the hatching mechanism of *Globodera* sp. *A. sativum* totally suppressed the hatching at *5* µl/ml followed by Eucalyptus and sage at 10 µl/ml. Chopped plants of marigold treatment have reduced population of PCN but radish and taatar plants had total PCN control.

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