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Centro de Sistemática e Ecologia, Departamento de Zoologia, Universidade de Coimbra, Coimbra, Portugal[1]

Department of Entomology and Nematology, IACR - Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, U.K.[2]

Departamento de Protecção de Plantas, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal[3] *and*

Departamento de Fitotecnia, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal[4]

A REVIEW OF POTATO CYST NEMATODES IN PORTUGAL

by

M. Susana N. De A. Santos[1], K. Evans[2], C. A. Abreu[3], F. F. Martins[4] and Isabel M. De O. Abrantes[1]

Summary. The potato crop in Portugal is grown on about 120,000 hectares (just over 3% of the total crop area) and, with other vegetables, represents about 20% of the value of agricultural production. The area under potatoes has increased in recent years but total production has remained steady at just over 1 m tonnes because average yields have declined. Some of this decline may be due to losses caused by potato cyst nematodes (PCN). Between 40 and 75% of potato fields were found to be infested with PCN in the main potato growing areas, with an average infestation level of about 20 eggs per g soil. Vila Real had fewest infested fields and the lowest overall population densities. Identification of the PCN species in 89 populations by isoelectric focusing, and checking the reaction of 60 of these populations to the H_1 and a further 20 to both the H_1 and H_2 resistance genes, showed that more than 50% were pure *Globodera rostochiensis* and controllable by cultivars containing the H_1 gene. Only five populations were pure *G. pallida* but at least two of these were able to produce only a few cysts on clone P55/7 (containing the H_2 gene) so may contain a proportion of pathotype Pal. Almost half the populations produced at least some cysts on Cara (containing the H_1 gene), indicating that many populations were mixtures of the two species. Nevertheless, correct deployment of H_1 resistant cultivars will play an important role in PCN management in Portugal.

The potato industry in Portugal

The Portuguese mainland covers 89,000 square kilometres and can be divided, as far as the potato crop is concerned, into three main zones - (i) the northerly part of the coast line and the littoral zone to the north of the River Tagus, which has mild to cool winters, mild summers, in duration and temperature, and more water available for irrigation, and is therefore the most intensively farmed, (ii) the inland area north of the Tagus valley, Trás-os-Montes and Beira Interior regions, where a range of cooler winters and dry summers is experienced; the region is so mountainous that only isolated areas can be used for crop production, and (iii) Alentejo, to the south of the Tagus River, where the spring and the summer are long, very dry and very hot, the potato crop being almost non-existent. About 50,000 square kilometres are in agricultural use, but crops are produced only on 36,000 square kilometres. Potatoes are grown on about 120,000 hectares (i.e. just over 3% of the total crop area) and, with other vegetables, represent about 20% of the value of agricultural production (Rolo *et al.*, 1986).

The area of potatoes grown each year has increased slowly from about 90,000 ha in the 1950s to 119,000 ha by 1990 (Table I). This increase reflects a need to maintain annual production at just over 1 m tonnes in the face of falling yields, which averaged over 12 t/ha in the 1950s but less than 9 t/ha in 1990. About 10% of the crop is early (harvested as early as March in the Algarve) but the rest is maincrop production and is concentrated in the north of the country.

Potato exports are negligible but considerable quantities of potato seed are imported. Domestic potato seed production is concentrated in the north of the district of Vila Real, which had 75% of the area in 1991. Total domestic seed production has increased quite a lot but still provides only a small proportion of the annual demand for certified seed: currently, about 2,000 tonnes of certified seed are produced each year in Portugal (Carvalho, 1992) but more than 70,000 tonnes have been imported in some recent years. Some of this imported seed is multiplied in Portugal but the great majority is sold directly to potato producers. Northern Ireland and The Netherlands are the main suppliers of imported seed, and the amounts imported depend on price of seed tubers and price of table potatoes in the previous season.

TABLE I - *Area of potatoes, total potato production and mean national yields on the Portuguese mainland, 1955-1990 (After INE, 1955-1990).*

Year		Area ('000 ha)	Production ('000 tonnes)	Mean Yield (tonnes/ha)
Average	1955-59	88	1071	12.1
Average	1960-64	104	1055	10.2
	1965	101	888	8.8
	1970	112	1220	10.9
	1975	107	1013	9.4
	1980	114	1118	9.8
	1985	126	1136	9.0
	1990	119	1038	8.7

Average yields of potatoes, 8.7 t/ha in 1990, are very low but much better yields can be attained, e.g. 25 t/ha in certain places. Yields are frequently low because of poor soils, adverse climate, or lack of irrigation, but potato blight and Colorado beetle also take their toll.

Potato cyst nematodes in Portugal

A less easily identified problem, but one which nevertheless can cause large yield losses, is that posed by potato cyst nematodes (PCN). These were first reported in Portugal in 1956 (Macara, 1963) but were probably introduced much earlier and have had the chance to spread throughout the potato growing districts. For this reason a project entitled "Survey of Golden Nematode of Potatoes and a Study of Control Techniques" was initiated at the beginning of 1984. The objectives were, over a period of four years, to survey the incidence of PCN in the principal potato-growing districts and to study techniques of integrated management which might reduce infestations to levels which would not affect potato yield. The project has been carried out by staff at the University of Trás-os-Montes e Alto Douro, Vila Real (Martins, 1985, 1986, 1987, 1990). Considerable progress has been made, with soil samples collected from potato fields throughout the districts of Vila Pouca de Aguiar (1984), Chaves (1985), Boticas (1985 and 1986), Vila Real (1985 and 1986), Valpaços (1986), Vinhais (1987) and Bragança (1987). The results of analysis of these samples are given in Table II. Potato cyst nematodes were found in a proportion of samples from all of the districts surveyed, ranging from 40% of the land area infested in Vila Real to 75% in Valpaços. In some villages all of the fields that were sampled were infested. Overall, infestation levels averaged 1.8 viable cysts per 10 g soil, which is equivalent to about 20

TABLE II - *Distribution of potato cyst nematode infestations within the main potato growing areas.*

District	Number of localities	Localities where all samples were infested	Number of fields	Area sampled (ha)	% Area infested	Viable cysts /10 g soil
Vila Pouca de Aguiar	35	10	311	86.2	71.9	1.9
Chaves	46	15	413	178.3	72.7	3.1
Boticas	32	5	262	86.3	51.1	1.3
Vila Real	17	0	124	33.1	40.0	1.3
Valpaços	39	11	413	255.3	74.6	2.2
Vinhais	27	3	313	78.1	42.7	1.6
Bragança	38	3	414	190.5	50.8	1.3

eggs/g soil. This is a moderate level of infestation but, of course, some infestations are much higher whilst some are lower. Many fields undoubtedly suffer serious economic loss. Also of concern is the very high proportion of fields (58% overall) which are infested. The infestation levels and the porportion of fields infested are reflections of how long ago the nematodes were introduced to an area and the frequency of cropping with potatoes since introduction. Thus, Vila Real appears to have become infested later than most areas, whilst Valpaços may have been one of the first areas to become infested.

Because resistant cultivars represent the best method of controlling PCN, the performance of a range of cultivars with the H_1 gene for resistance was checked at several locations. Several of these cultivars produced good yields and will be useful additions to the traditionally grown cultivars where nematode infestations are of the *Globodera rostochiensis* (Wollenweber) Behrens pathotype unable to multiply on them. Following the initial tests, a much greater number of nematode populations were tested at the University of Coimbra in 1987. These populations came from the University of Trás-os-Montes e Alto Douro survey, and 92 were chosen to provide populations representative of all the areas sampled to the end of 1986. In order to provide sufficient material for all of the tests it was necessary to choose samples from populations which contained large numbers of cysts, and this is reflected in the large egg population densities per unit weight of soil found in them, ranging from 71 eggs/g soil in Figueira de Castelo Rodrigo to 267 eggs/g soil in Vila Pouca de Aguiar (Table III). Isoelectric focusing (IEF) of the water soluble proteins from whole cysts reveals species-specific bands in PCN and allows populations to be classified as *G. rostochiensis*, *G. pallida* (Stone) Behrens or mixtures of the two (Fleming and Marks, 1983). Table III shows the classification of 89 of the 92 populations, with 55 pure populations of *G. rostochiensis*, 5 of *G. pallida* and 29 mixtures.

The conclusions of the EPPO Workshop on Cyst Nematodes (Anon., 1985) include the statement that only one distinct pathotype of each of the species of PCN can be recognised, depending on reaction to the single major resistance genes H_1 and H_2. Because one pathotype of each species can be positively identified, it follows that two further "pathotypes" can be defined by default. The frequency with which these pathotypes occur is important because it determines the usefulness of cultivars containing genes H_1 and H_2 in nematode management

TABLE III - *Potato cyst nematode populations chosen for study at the University of Coimbra.*

District	Number of populations	Mean no. of eggs /g soil	Number of populations used for IEF*	Number of populations identified by IEF		
				G. rostochiensis	*G. pallida*	Mixture
Vila Pouca de Aguiar	18	267	18	10	2	6
Chaves	31	150	29	22	0	7
Boticas	9	91	9	2	0	7
Vila Real	5	213	5	1	0	4
Valpaços	28	130	27	20	2	5
Figueira de Castelo Rodrigo	1	71	1	0	1	0
Total	92		89	55	5	29

* IEF = Isoelectric focusing.

strategies. Therefore, 80 populations from the 92 listed in Table III were chosen for a differential test using a non-resistant potato cultivar (Désirée), a cultivar with gene H_1 (Cara) and a clone with gene H_2 (P55/7). Only 20 populations could be tested against genes H_1 and H_2, the other 60 being tested against gene H_1 only. For the tests, potato plants were grown from single sprouts in pots which held 350 g of sterile sandy loam soil. Sufficient cysts to give an initial nematode density of 5 eggs/g soil were enclosed in small pieces of polyester voile, so that any new cysts which formed could be separated from the cysts forming the inoculum. There were three replicates of each treatment combination and the exterior of the root ball was examined 10 weeks after planting to count the numbers of females which were visible. At this stage of development, females of *G. rostochiensis* are yellow whilst those of *G. pallida* are white. Twenty weeks after planting the tops were removed from the plants, the soil dried and sieved and the new cysts that had formed were extracted. The results of the root ball examinations and cyst counts, along with the results from the IEF tests, are given in Table IV (Vila Pouca de Aguiar), Table V (Chaves), Table VI (Boticas, Vila Real and Figueira de Castelo Rodrigo), and Table VII (Valpaços).

In the pathotype test, all populations produced some cysts on Désirée but the numbers varied between 7 and 398 per plant (mean = 139) despite the standard inoculum of 5 eggs/g soil. Much of the variation was probably due to low viability in some populations and additional variation may have been caused by high soil temperatures close to the limit for nematode survival during the pot experiment. Despite this variation some interesting observations can be made from the results:

(i) The overall production of females visible on the root ball surface at 10 weeks relative to the number of cysts extracted at the end of the experiment was only 12.8%. This figure varied between clones, being only 4.1 and 7.0% in Cara and P55/7, respectively, but 14.1% in Désirée. Using counts from root balls could, therefore, lead to erroneous conclusions: from populations against which both clones were tested, P55/7 produced only 25.5% of the number of females on root balls of Désirée but 55.7% of the number of cysts.

(ii) Four of the five populations classed as pure *G. pallida* by IEF produced many cysts on

Cara (over 78% of the number on Désirée) but one (Figueira de Castelo Rodrigo) produced none. However, of the three which were tested on P55/7, that from Figueira de Castelo Rodrigo was the only one producing as many cysts on P55/7 as on Désirée: the other two produced many fewer. This suggests that at least two of the pure *G. pallida* populations contain a proportion of pathotype Pal.

(iii) Most populations produced no or very few cysts on Cara, supporting the IEF results that most infestations are *G. rostochiensis*. However, some 15 populations produced, on Cara, more than 10% of the number of cysts on Désirée; 34 populations contained at least some *G. pallida* according to IEF results whilst 39 produced at least some cysts on Cara.

(iv) Some populations produced more cysts on P55/7 than on Désirée but 12 out of 20 tested produced less than 50% of the numbers on Désirée. Of these 12, seven contained *G. pallida* (according to IEF) and the reduced numbers of cysts on P55/7 could have been due to the H2 gene preventing reproduction of pathotype Pal of *G. pallida*. The remaining 5 populations were pure *G. rostochiensis* according to IEF and it is therefore possible that P55/7 contains a resistance gene or genes capable of reducing multiplication of some *G. rostochiensis* populations.

TABLE IV - *Species determination by IEF and pathotype determination by differential clones for populations from Vila Pouca de Aguiar.*

Population Code	% by IEF of		Females on root balls of			Cysts/plant of		
	G. rostochiensis	*G. pallida*	Cara	Désirée	P55/7	Cara	Désirée	P55/7
483	100	0	0	6	1	1	58	10
510	95	5	0	27	–*	1	364	–
514	100	0	0	59	–	3	358	–
514/330	100	0	0	9	–	4	100	–
556	70	30	0	74	–	1	398	–
561	100	0	0	56	–	68	336	–
562	60	40	0	49	–	3	298	–
809	70	30	0	6	0	0	18	7
810	90	10	0	4	–	7	43	–
814	100	0	0	8	–	7	51	–
2122	65	35	0	61	10	1	330	162
2133	100	0	0	37	24	0	163	138
2171	100	0	0	5	–	0	99	–
2173	100	0	0	2	–	0	41	–
2174	100	0	0	0	–	0	30	–
2194	0	100	1	10	–	82	147	–
2195	0	100	10	31	3	138	127	22

* - = populations not tested.

TABLE V - *Species determination by IEF and pathotype determination by differential clones for populations from Chaves.*

Population Code	% by IEF of		Females on root balls of			Cysts/plant of		
	G. rostochiensis	*G. pallida*	Cara	Désirée	P55/7	Cara	Désirée	P55/7
688	80	20	0	15	3	0	132	42
688/313	100	0	0	2	-*	1	40	-
705	100	0	0	1	-	3	21	-
713	100	0	0	13	-	4	135	-
720	95	5	2	53	-	16	360	-
725	100	0	0	48	3	1	267	144
767	100	0	1	3	-	1	61	-
774	95	5	0	12	1	0	98	28
798	100	0	0	6	0	1	51	10
877	100	0	0	5	-	0	58	-
930	100	0	0	40	5	0	256	122
930/330	100	0	0	5	-	1	75	-
932	70	30	0	24	-	0	140	-
933/340	100	0	0	1	-	0	17	-
934	100	0	0	32	-	0	308	-
934/440	100	0	0	2	-	0	54	-
936	100	0	0	0	-	1	18	-
939	100	0	0	33	-	0	122	-
940	90	10	0	49	-	0	273	-
940/312	100	0	0	1	-	2	7	-
946	50	50	0	39	10	0	223	94
946/312	40	60	0	0	-	3	12	-
947	100	0	0	0	0	0	13	2

* - = populations not tested.

Conclusions

It is clear that there are many severe infestations of PCN in the potato production areas of Portugal. Considerable yield loss is, undoubtedly, caused by these infestations. About 50% of them seem to be pure *G. rostochiensis* against which the H_1 resistance gene is effective. Cultivars with this gene will therefore be invaluable for control of the nematodes in compliance with the EU directive on PCN (Anon., 1969). The use of appropriate nematicides in infested areas would also comply with the directive, but average yields in Portugal may be too low to support the use of currently available nematicides, which are all expensive. The most important aspect of the EU directive relates to seed potato production, which is not allowed on infested land. Proper testing procedures must be used to ensure that seed production areas are

nematode-free and are kept so by appropriate management strategies.

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TABLE VI - *Species determination by IEF and pathotype determination by differential clones for populations from Boticas, Vila Real and Figueira de Castelo Rodrigo.*

Population Code	% by IEF of		Females on root balls of			Cysts/plant of		
	G. rostochiensis	*G. pallida*	Cara	Désirée	P55/7	Cara	Désirée	P55/7
Boticas								
1816	40	60	4	54	–*	40	247	–
1817	80	20	0	17	8	0	149	150
1877	90	10	0	10	–	0	67	–
1896	90	10	0	14	–	1	114	–
1903	50	50	1	33	–	24	219	–
1904	70	30	2	36	–	25	177	–
1905	100	0	0	4	–	0	33	–
1914	95	5	0	6	9	0	35	36
Vila Real								
2076	100	0	0	2	5	0	34	61
2077	85	15	0	48	–	3	234	–
2078	90	10	0	29	–	2	198	–
2080	80	20	0	53	–	0	391	–
2083	75	25	0	0	–	0	14	–
Fgueira de Castelo Rodrigo								
710	0	100	0	2	0	0	32	35

* - = populations not tested.

TABLE VII - *Species determination by IEF and pathotype determination by differential clones for populations from Valpaços.*

Population Code	% by IEF of		Females on root balls of			Cysts/plant of		
	G. rostochiensis	*G. pallida*	Cara	Désirée	P55/7	Cara	Désirée	P55/7
2554	100	0	0	17	–*	1	58	–
2556	0	100	3	2	1	82	74	21
2557	100	0	0	35	–	32	158	–
2559	100	0	0	1	–	1	38	–
2563	90	10	0	0	–	3	28	–
2565	100	0	0	0	0	0	55	7
2571	100	0	0	6	–	0	76	–
2572	100	0	0	7	–	0	46	–
2578	100	0	0	2	0	0	25	44
2618	100	0	0	7	–	1	80	–
2630	95	5	0	4	–	0	85	–
2647	100	0	0	1	–	0	37	–
2649	100	0	0	0	–	0	21	–
2650	90	10	0	0	–	0	16	–
2675	100	0	0	9	4	0	87	105
2699	95	5	0	34	–	1	155	–
2719	100	0	0	0	–	0	13	–
2724	90	10	0	12	–	0	104	–
2739	100	0	0	21	–	2	166	–
2763	100	0	0	3	–	1	38	–
2780	0	100	0	0	–	17	60	–
2786	100	0	0	1	–	0	43	–
2802	100	0	0	2	–	0	31	–
2894	100	0	0	4	–	0	58	–
2907	100	0	0	2	–	0	51	–
2943	100	0	0	1	–	0	15	–

* - = populations not tested.

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