# AHDB Potatoes Research Project: Provision of Content for a PCN Management Guide (No: 1240001)

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## Potato Cyst Nematodes (PCN) - Their Characteristics and Guide to Management

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## *Chapter 1. Introduction*

The spread of *Globodera pallida*, and the withdrawal of many pesticide controls, has necessitated a major reassessment of criteria for control of both species of PCN. Since the MAFF guide to PCN Management was issued in 1999, there have been several advances in knowledge of PCN development, the development of potato varieties resistant to *G. pallida* as well as *G. rostochiensis*, and other new tools to assist in reducing population levels before the next crop. The biology and management of PCN is a complex subject, and it is clearly apparent that the characteristics of PCN differ not only according to species, but also according to local environmental conditions; thus this Reference Guide will present current knowledge, but local observations are invaluable for making the right decisions.

The aim of this Reference Guide is to put science into practice and provide guidelines for the management of potato cyst nematodes (PCN) and so improve production levels in the UK. Commercial demands may have to be reviewed if the long-term control of PCN and the future of the industry is to be sustained, especially when evidence is accumulating that viable eggs can remain in cysts for 40 years or more.

An earlier guide for the management of PCN produced by MAFF in 1999 (Lane & Trudgill, 1999) was in response to a need to manage the emergence of *G. pallida,* as it is more troublesome to control than *G. rostochiensis*. The former is now the predominant species, and a reduction in the range of nematicides/nematistats to control it has demanded a more sustainable approach if the area of potato growing land is to be protected. For decades, PCN have remained a major pest of potatoes in the UK, and there are new records worldwide each year. In 2010, the EU published a new PCN Control Directive (EU Council Directive 2007/33/EC - Anon, 2007), to spearhead a more collaborative approach to the management of this pest in Europe (Hockland, 2010). The development of molecular science has increased our understanding of PCN, but several problems, such as the increasing importance of *G. pallida,* and availability of commercially-viable resistant varieties, remain.

There are several recommended management tools integrated into this Reference Guide. Potato varieties showing resistance to the PCN species are key components. However, to date, the commercial popularity of certain varieties for the fresh sector or processing has

assumed a greater importance than using a PCN resistant variety and this has been largely responsible for an increase in PCN populations. This situation must receive greater attention from potato processors and retail outlets, whose involvement in PCN management is critical.

The identification of species is an essential precursor to choice of variety. Any control programme should allow a tailored approach to every field, yet it seems that many management strategies are based on assumptions of the species present. Knowledge of the life cycle of the particular PCN species present would facilitate plans regarding the choice of variety, length of rotation, a realistic assessment of the likely effectiveness of chemical products, and risk of more than one generation occurring.

Whilst sampling and detection methods are essential to provide the raw data for determining the optimal path for management, procedures vary nationally, and are notorious for being subject to variation and error. This Reference Guide advocates an approach that uses the detection of any viable cyst as the trigger for action. However, there is no ‘silver bullet’. Sampling techniques should be carefully considered to maximise the chances of detection in a field, bearing in mind cropping history and likely pathways for spread; levels of infestation should be monitored and the results used in decision-making for management methods in a particular field.

The decrease in the availability of chemical tools has increased attention into research for biological control, but to date these remain to be developed for commercial use. The most important contribution a grower or buyer can make to sustainable PCN management in infested fields is to use a potato variety of the required resistance grading; this Reference Guide emphasises that importance. However, there is also potential for trap crops and biofumigants to become an integral part of control measures.

Above all, it is clear that the management of PCN is a complex subject, and requires detailed knowledge of the characteristics of the local PCN population and environmental conditions to develop a successful strategy for any particular field.

## *Chapter 2. History: Origin and Spread*

Knowledge of the origin and pathways of spread of both PCN species can contribute to an understanding of those factors that will facilitate its management.

The PCN species considered in this Reference Guide are closely related species, namely *Globodera pallida* (Stone, 1973) and *G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959, which co-evolved with the wild potato in South America several hundred thousand years ago (Stone, 1979). Molecular studies are providing increasing evidence that the ancestors of populations in Europe probably came from a few introductions, probably from southern Peru in the 19th century. Several expeditions were made then to central and south America in order to procure new, cultivated potato varieties resistant to potato blight (*Phytophthora infestans*) (Turner & Evans, 1998).

However, some of the introductions into Europe may have been via routes other than as contaminants of potato tubers. Inagaki & Kegasawa (1973) found viable cysts of *G. rostochiensis* in Peruvian guano, imported into Japan from deposits located off the coast of Peru. It seems most likely that the guano was shipped in PCN-contaminated potato sacks, and may have been introduced to Europe this way from the 1840s onwards, explaining the presence of the two species here today. Historical records suggest that *G. rostochiensis* may have arrived in the USA in soil on military or agricultural equipment (Hockland et al. 2013), thus illustrating the need to be vigilant on all potential pathways.

A recent report from the European Food Safety Authority (EFSA) Panel on Plant Health delivered a scientific opinion on different risks posed by European and non-European populations of PCN to potatoes and other solanaceous plants in the EU and on the effectiveness of current control measures (EFSA Panel on Plant Health (PLH) (2012)). Although PCN is widespread in the EU, breeders have only developed varieties that are resistant to the small number of genotypes that are present in Europe. These genotypes represent a minor subset of the gene pool and virulence that is present in South America. As new South American genotypes are very likely to have a similar potential for establishment and spread as existing European genotypes, the potato varieties currently grown in Europe are thus vulnerable to attack by populations with new characteristics. As resistant varieties

take a very long time to develop, a new introduction from South America or elsewhere would have major economic consequences.

## *Chapter 3. Biology and Distribution of PCN species, including Life Cycle, Population Dynamics and factors influencing them*

A knowledge of the biology and distribution of each PCN species will enable the sustainable development of control measures that will reduce the spread and importance of economically damaging populations.

### *3.1.Species and Distribution*

*Fig. 1. Maturing females and cysts of Globodera rostochiensis (Courtesy Fera)*

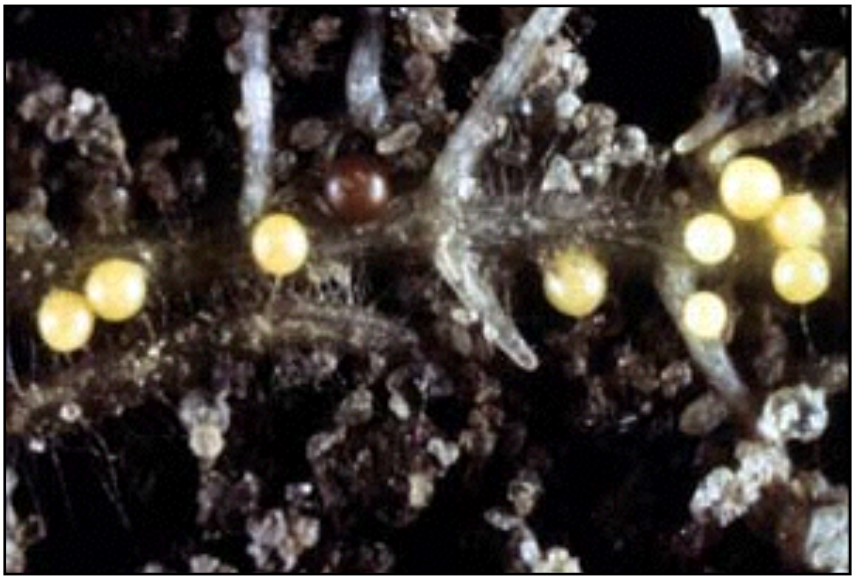
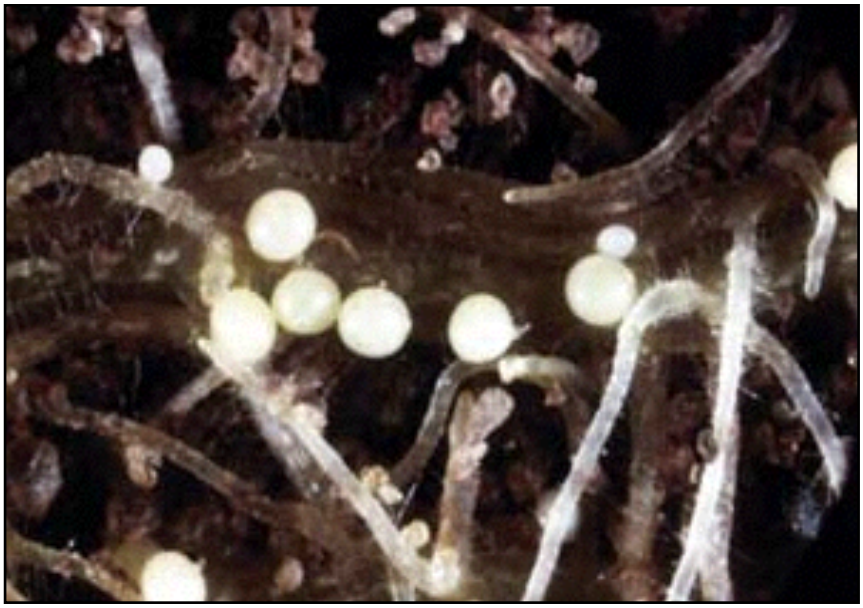


*Fig. 2. Maturing females and cysts of Globodera pallida (Courtesy Fera)*



Accurate knowledge of the PCN species infesting fields is thus a basic premise for devising sound integrated pest management programmes, primarily because of the differences in biology which will affect the choice of resistant varieties and lengths of crop rotation, but also because it will lead to more effective use of other management tools (Palomares et al. 2014).

The PCN species belong to the group of cyst nematodes that have round, or very slightly pear-shaped cysts (genus *Globodera*). There are currently two species that infest potato in the UK, namely *G. pallida* and *G. rostochiensis*. There is evidence of marked physiological differences between them at certain phases of their life cycles. In terms of hatching, development and persistence, including the utilisation of fat reserves, *G. pallida* is the species better adapted to low temperatures and this is consistent with its ability to gain an advantage over *G. rostochiensis* in cool soils. The prolonged hatch and persistence of *G. pallida* may also ensure that root growth is less affected, thus reducing intraspecific competition between females (Perry, 1998).

Minnis et al. (2002) remains the most recent, statistically valid, report of the distribution of the PCN species in England and Wales. Their survey of potato farms in England and Wales showed PCN present in 64% of sites sampled, with 67% of the populations being recorded as *G. pallida*, 8% *G. rostochiensis*, and 25% being a mixture of the two species. Ware survey work as prompted by the recent EU Directive was initially done on an ad hoc basis and hence cannot be considered as truly representative of the current situation. Nevertheless, it does provide support for the widespread presence of *G. pallida*, and the persistence of *G. rostochiensis*.

*Fig. 3. Sites where PCN cysts were found in a 1997-1998 survey by Minnis et al. (2002).*

*Fig. 4. Sites which contained G. pallida cysts in a 1997-1998 survey by Minnis et al. (2002).*

*Fig. 5. Sites which contained G. rostochiensis cysts in a 1997-1998 survey by Minnis et al. (2002).*

Recent anecdotal evidence from ware surveys, statutory and commercial laboratories confirm *G. pallida* continues to be the dominant species in England and Wales, and in ware potato land in Scotland. A significant proportion of fields in England and Wales contain a mixture of *G. pallida* and *G. rostochiensis*, and a few fields are infested with *G. rostochiensis* only. The latter species is still the dominant species in all seed growing land in Scotland, with the exception of Angus where potatoes have typically been grown most intensively (Jon Pickup, personal communication.).

*G. achilleae* is a *Globodera* species native to the UK and it is occasionally identified in laboratories; it does not parasitise potatoes but feeds on Yarrow (*Achillea millefolium*), so it is important it is not confused with the PCN species. Recent rDNA work by Subbotin et al. (2011) supports the synonymisation of this species with *G. millefolii,* and of *G. hypolysi* with *G. artemisiae,* which has been recorded in Europe. The latter species did not breed on potato in an outdoor pot experiment (Dobosz et al. 2009).

Previously unknown species of *Globodera* have recently been described in the Americas, such as *G. ellingtonae,* a species recently detected in Oregon, USA and Argentina, which has been shown to also feed on potato (Chronis et al. 2014; Lax et al. 2014; Zasada et al. 2013; Handoo et al. 2012).

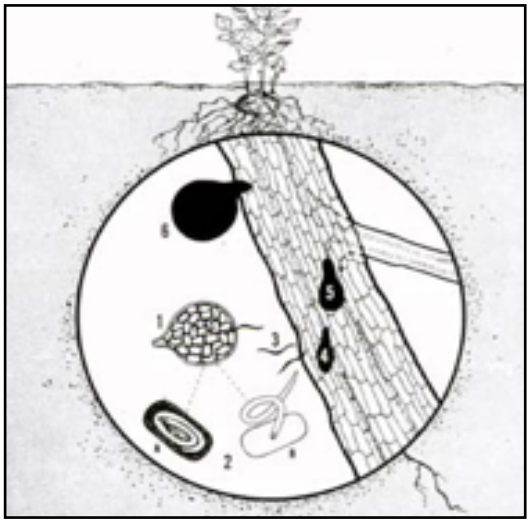
Occasionally, morphological variants of PCN have been recorded in the UK; Reid & Pickup (2005) recorded a cyst that gave hybrid patterns showing elements of both *G. pallida* and *G. rostochiensis,* and more research is required to see if such variants are biologically different. As the use of molecular-based tools improves, so new records of PCN are being published from Europe and elsewhere (Njezic et al. 2014; Grubisic et al. 2013; Blok & Phillips, 2012) and others).

### *3.2 Life Cycle*

#### *3.2.1. Introduction*

Field observations of the biology and life cycle of the PCN species depend on a sound knowledge of the species present. But other factors, such as the variety being grown, soil type and aspect may also be significant, so non-UK research data should be used with some caution, as it might not be applicable to PCN reproducing on varieties cropped here e.g. Belgian work on life cycle which has been done on the cv. Eerstling (susceptible to *G. pallida*).

*Fig. 6. Life cycle of PCN. 1. Juvenile nematode leaving cyst. 2A. Appearance of juvenile in egg. 2B. Juvenile nematode hatching from egg. 3. Juveniles entering potato root. 4 and 5. Immature females inside root. 6. Mature female. (Anon)*

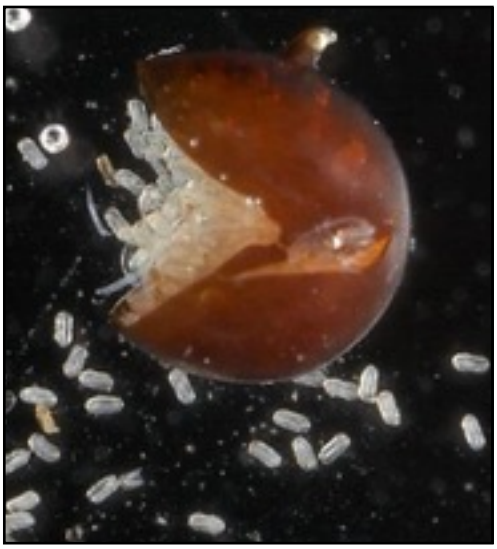


The PCN cyst is actually the dead body of the female, which may contain up to 600 eggs (Anon, 1989). It is the main means of survival and spread for the species. It has a porous shell that probably functions as a way of keeping a population of nematodes together for spreading, an essential feature for a sexually-reproducing organism. The developing juveniles are protected from desiccation and parasites by their eggshell, which enhances their ability to remain dormant for many years (John Jones, personal communication). The cysts are usually about 0.5mm in diameter and hence just visible to the unaided eye; those of *G. rostochiensis* have an early yellow or gold phase (hence 'the golden potato cyst nematode'), whilst those of *G. pallida* have a cream phase (hence 'pale potato cyst nematode'). These colours are only visible for a short time before the females die and both form the hard, dark brown cysts. They are primarily root parasites, although they may also occur on underground stems, such as stolons and tubers (Oostenbrink, 1950).

### *3.2.2. Hatching*

PCN juveniles do not hatch from their eggs all at once; only a proportion do so gradually in a season and others even more gradually over years and decades, primarily in response to potato root diffusates. What determines the gradual release over years is not fully understood, but it is of critical importance in understanding the complex management of PCN. *G.pallida* is able to hatch at lower temperatures (4°C) and has a longer hatching period than *G. rostochiensis* (which starts to hatch at 6°C), but there is also evidence that hatching may differ between populations of the same species, as an adaptation to local climate conditions.

*Fig. 7. A mature cyst split to reveal eggs and hatching juveniles (courtesy Fera)*



Each egg contains a juvenile nematode; one moult occurs in the egg so that on hatching the juveniles are in their second stage (J2). In the spring, upto one third of the eggs may hatch spontaneously, even in the absence of a host plant, and the new juveniles may persist in the soil for several weeks (Anon, 1989). The failure of the remaining eggs to hatch in any one year is seen as part of the PCN survival strategy for future years (Turner & Evans, 1998); indeed, viable juveniles can remain dormant within cysts in soil for upto 40 years or longer before they emerge from the eggs.

The hatching process itself continues to be investigated (Perry et al. 2013). Trehalose, a sugar of the disaccharide class, is contained in the perivitelline fluid which surrounds the juvenile in the egg. When the permeability of the egg shell is changed by hatching factors, the trehalose leaves the egg and permits an influx of water and subsequent rehydration of the juvenile to a water content commensurate with a resumption of metabolism; the resultant change in turgor pressure within the egg also triggers muscular activity. The presence of trehalose in eggs containing unhatched, viable juveniles (or J2s) has been used as the basis for a viability assay for *G. rostochiensis* and *G. pallida* (van den Elsen et al. 2012).

Hatching is also affected by abiotic factors such as temperature, aeration and moisture levels, so it is a complex picture (Perry, 1998), but the major influencing factor in any one season is the root diffusate emanating from the potato root, most actively from the root tips (Rawsthorne & Brodie, 1986). There are differences between *G. rostochiensis* and *G. pall-*

*ida* in their hatching response, as they react differently to the plant chemicals present in the potato root diffusate. *G. rostochiensis* hatches faster in response to them, behaviour that might relate to the adaptation of this species to its particular ancestral hosts in the Andes (Perry et al. 2013). *G. pallida* shows a greater response to the plant chemicals produced in later stages of growth of the potato plant and laboratory tests have shown that *G. pallida* has a much slower initial rate of hatch and utilises its fat (lipid) reserves more slowly than *G. rostochiensis* (Robinson et al. 1987a;1987b).

*G. pallida* appears to have a longer hatching period than *G. rostochiensis,* often quoted as 18 weeks compared to 12 weeks, thus cysts can potentially be produced through much of the summer, even if they are from one generation in the spring. This means that anecdotal evidence of a 'second generation', and the viability of juveniles from such a generation, needs to be investigated further to ensure the late emergence of cysts is not just a late generation. In contrast, observations on the hatching behaviour of both species of PCN in a field in Ireland revealed there was no significant difference in hatch times (Ryan & Devine, 2005). Hatches in excess of 80% for both species were recorded 8 weeks after plant emergence between 10-40cm depth and distances 10-50 cm around the host plants. This would suggest more field observations are needed to gather evidence for environmental factors that might affect hatching.

Research by Turner et al. (2009), showed that root diffusates from different wild *Solanum* species elicited different responses in different populations of *G. pallida*, but not *G. rostochiensis,* leading to a hypothesis that the differences were due to different coevolutionary histories of the nematodes and their *Solanum* hosts in South America. The fractionation of potato root diffuse revealed the presence of more than 10 hatching factors with activity towards PCN. The total chemical synthesis of Solanoeclepin A, a key component of the hatching factors, has been reported (Tanino et al. 2011). As well as plant-derived hatching factors, there is increasing evidence for a role for microbial hatching factors for cyst nematodes in the soil (Deliopoulos et al. 2011). Two other classes of hatching chemicals have been identified in potato root diffusate, namely hatch inhibitors (HIs), which are produced early in the season, and hatching factor stimulants (HFSs), which are produced later, resulting in an initial net inhibition of hatch while the roots of the new plants become established (Perry et al. 2013). Subsequently, an increase in the HFS:HI ratio stimulates the juveniles to hatch (Byrne et al. 1998).

Hatching factors in root diffusates are highly mobile in the soil, with activity towards *Globodera* being detected up to 80cm from the potato root zone (Perry et al. 2013). Substantial hatches of 82-88% were also recorded at a depth of 20cm in the furrow at harvest. However, monitoring the rate of spontaneous hatch in the spring and summer months without a crop host (20 weeks) revealed there was a significant difference in the rate of spontaneous hatching, with *G. rostochiensis* hatching in greater numbers over the first 14 weeks than *G. pallida*, but by mid-August the mean spontaneous hatch for both PCN species within the top 30cm of soil was found to be 37%; whether this was a second generation is not reported.

It is well documented that there is great variation in the rates of hatching between different populations of *G. pallida* and *G.rostochiensis* from different geographic locations (Evans, 1983). Byrne (1997) examined the hatching responses of the two species of PCN from the UK and The Netherlands in laboratory experiments and found that there were greater correlations between the hatching responses of the different species from similar geographic origins than there were between the same PCN species from different geographic origin. Thus the use of research data from different geographical regions needs to be used with caution. Different varieties may also affect hatching response, but the available evidence suggests the effect is insignificant.

*3.2.3.How juveniles become established in a host*

Like other plant pathogens, PCN needs to suppress host defence responses in order to invade it. This is a challenge, as plants have developed highly sophisticated defence mechanisms to provide protection from invading pathogens. The unravelling of mechanisms that nematodes use to counteract them, such as the production of their own effectors (or plant-chemical imitators) to enable them to suppress a plant's programmed cell death, are gradually being elucidated. When the juveniles reach a host plant they invade it, secreting cell wall modifying proteins (cell-wall-degrading enzymes) (Rehman et al. 2009; Kudla et al. 2007). They then settle down in the cortex near the conducting tissue of the root or maybe later a tuber initial. It is now generally accepted that successful invasion by the nematode depends on it secreting an elaborate repertoire of effector proteins that suppress the damage-triggered immune responses of the host and prevent cell death (Ali et al. 2015; Mei et al. 2015; Lozano-Torres et al. 2014; Thorpe et al. 2014; Jones et al. 2009; and numerous other papers). A wide range of effectors have been identified and characterised from PCN and other cyst nematode species. These effectors also facilitate migra-

tion within the plant and induce the formation of a syncytium, a multinucleate food-transfer cell, in which the head of the nematode remains embedded, as it feeds, until maturity. It depends on this syncytium for all nutrients required to develop to the adult stage (Thorpe et al. 2014).

PCN and other cyst nematodes have been shown to produce effectors that mimic proteins produced by the plant itself, an amazing evolution by the nematodes to facilitate their parasitism of plants. A better understanding of this extraordinary example of molecular mimicry is advancing our knowledge of plant-nematode interactions. CLAVATA3/ESR (CLE)-like peptides (*e.g.* Chen et al. 2010) are one such group of effectors that have diverse roles in the control of plant growth and development, and a role in controlling feeding site formation has been suggested. However, the details of the mechanisms underlying this process remain unclear. For example, modulation of auxin transport is known to be essential for formation of syncytia (Grunewald et al. 2009) and an effector has been identified from soybean cyst nematode that interacts with an auxin transport protein (Lee et al. 2011). More recently it has been shown that cyst nematodes synthesise and secrete cytokinins, and that this process is essential for feeding site formation (Siddique et al. 2015).

When PCN interacts with resistant varieties, however, other complex interactions come into play. Specific pathogen-produced effector proteins, encoded by effector or virulence (Avr) genes, are recognised by potato plant *R* genes, triggering a cascade of defence responses (Bent & Mackey, 2007). There are other mechanisms employed by varieties with varying levels of resistance; visual signs include a delay and degeneration in the formation of nematode-induced syncytia, and a delay in the development of females, compared to susceptible varieties (Palomares-Rius et al. 2012). Some recent work involves research to investigate why syncytia sometimes fail to develop in roots (thus preventing the juvenile nematode from further development). Dabrowska-Bronk et al. (2015), in an investigation into RNA interference or the inducible over-expression of nematocidal genes, describe new candidate genes which show promise, including those that play important roles in the development of nematode-induced syncytia. For those wanting to know more on nematode effectors, a useful ‘mini-review’ was produced by Quentin et al. (2013).

Castelli et al. (2006) recorded the invasion of *G. pallida* on *S. canasense*, and found that J2s rarely induced a functional syncytium upon which to feed, and when they did the syncytia were markedly smaller than might be expected, and necrotic responses, usually as-

sociated with resistant plants, were not observed. Infestation by PCN on most resistant potato varieties also appears to result in an increased male to female ratio (Castelli et al. 2005).

As research into plant-nematode interactions continues, so alternative strategies for production of resistant varieties are offered which may result in a decrease in levels of PCN, but whether such varieties will satisfy commercial markets remains to be seen. Investigating the genes in PCN which assist its parasitism in potatoes, such as those that are responsible for the effector proteins mentioned earlier, may lead to broader mechanisms of resistance, by suppressing the relevant genes in the nematodes. Also, resistance based on the expression of proteinase inhibitors to disrupt nematode digestion may emerge as a promising route (Fuller et al. 2008).

Whilst many free-living plant-parasitic nematodes are able to at least penetrate roots to feed, it is believed they are unable to penetrate the skin of mature tubers of some varieties. It is not known whether the same applies to PCN juveniles. Certainly, especially in heavy infestations, juveniles become embedded in tubers and are seen on maturity as cysts, most commonly in protected positions around the ‘eyes’ or buds on the tuber (Dunnett, 1957). Thus an investigation into this behaviour, combined with an estimate of the timing of formation of tubers for a range of varieties, would allow an assessment of whether cysts on tubers may be the result of a prolonged hatch of juveniles onto forming tubers, or a true second generation.

*3.2.4. Maturity, death and the next generation*

The sex ratio in PCN is determined by its environment. Juveniles that are able to induce a large feeding site and obtain a good food supply become female while those that induce a smaller or restricted feeding site that does not reach the vascular tissue become males. Consequently when the juveniles are severely crowded, or under nutrient stress, the syncytia are smaller and this leads to a greater proportion of males; when there is no overcrowding a larger proportion of juveniles become females (Turner & Evans, 1998).

When the female PCN is mature she has become so enlarged that she ruptures the root cortex and is exposed in the rhizosphere, but she continues to feed from the syncytium during egg production. Pheromones from the females attract adult vermiform males, that have exited the roots. A single female may mate with multiple males (Eves-van den Akker

et al. 2015). After fertilisation and subsequent death of the female, her body forms a cyst that contains the eggs. Unhatched juveniles of PCN remain dormant (quiescent) during adverse conditions, until stimulated to hatch by potato root diffuse. However, a certain percentage of juveniles remain inside their eggs and remain in a dormant state (diapause) until specific requirements have been met, even if favourable conditions return. This ability of PCN to remain dormant and viable for up to 40 years is a key survival mechanism and risk to potato cropping that needs to be borne in mind when developing management strategies.

*3.2.5. Rate of decline*

The rate of decline of PCN generally refers to the rate of spontaneous hatching from each cyst each year, and not the natural mortality rate, which Devine et al. (1999), found reduced the number of viable eggs by about 10%. This rate was positively correlated with field soil temperature. As PCN will only multiply on potato crops in arable land in the UK, growing them infrequently decreases the population between crops and can be a major tool in PCN management.

Spontaneous hatch is influenced by many biotic and abiotic factors in the field, thus it is difficult to calculate accurate decline rates for PCN over a whole field, and hence difficult to provide growers with site-specific computer programs (Ryan & Devine, 2005). Rate of decline is greatly influenced by climate, field aspect and soil type (Anon, 1989) with rate of decline slower in organic soils than on mineral soils; a decline rate of 32% has been recorded for PCN populations in silt, clay and black fen soils (Cole & Howard, 1962a), but up to 60% in sandy soils (Cole & Howard, 1962b). Temperature and moisture content are also thought to be important (Turner & Evans, 1998); in cold soils the annual decline due to hatch may be as low as 18% (Grainger, 1964). This variation is reflected in Table 1. An important observation is that the rate of decline for *G. pallida* is generally slower than that for *G. rostochiensis*, but nevertheless hatching occurs more readily than *G. rostochiensis* in cooler soils, whilst the latter hatches more readily in warmer soils. This means *G. pallida* will start hatching earlier in the spring. All these factors support the recommendation to identify the particular species infesting fields, which will thus influence many decisions, such as the length of rotation.

*Table 1. Natural decline rates per year reported for PCN in general, and for G. pallida and G. rostochiensis in the UK.*

| | PCN in general | *G. pallida* | *G. rostochiensis* |
|---|---|---|---|
| | | | |
| Trudgill et al. 2014 | | 15% - 33.5% (mean 26%) | |
| Lane & Trudgill, 1999 | 12% | 20% | 30% |
| Whitehead & Turner, 1998 | 20% - 49% | | |
| Stone et al. 1973 | | 15% - 24% | |
| Cole and Howard, 1962a | | | 32% |
| Cole and Howard, 1962b | 60% | | |
| Cooper, 1953; Grainger, 1964,1951 | | | 18% - 33% |

Much of the early data on decline rates is difficult to interpret now because population estimates were based on cysts and not eggs, and the age and species structure of the populations was often not defined (Defra Report HH3111TPO_1748_FRP, 2003). Trudgill et al. (2014) recorded major differences in decline rates between sites. This may be due to several factors, such as soil type. According to earlier data, on a mineral soil heavily infested with *G. pallida* (100 eggs/g soil), it may take 13 years for the population to decline, without the use of nematicides or resistant varieties, to a level that is safe to grow a susceptible potato crop. Several agronomists have also recorded variable decline rates in the field, such as from 10% - 50% per year (A. Barker, personal communication).

Stone et al. (1973) demonstrated that non-host cropping with cereals, grasses and a range of horticultural crops had no effect on the rates of decline of *G. pallida*, which varied between 15 and 24% per annum over a 7 year period. However, regular cultivation of *Solanum sisymbriifolium* (sticky nightshade, SN) in an infested soil has been shown to increase decline rates (Turner, 1996). Volunteer potato plants at densities greater than a plant per $m^{-2}$ can maintain or increase PCN abundance locally where infestations in a field generally appear to be low.

Rates of decline have also been shown to be variable elsewhere in Europe. Observations on *G. pallida* in Serbia (Radivojevic & Grujic, 2010) showed that over four years the numbers of viable eggs and juveniles in the cysts declined slowly from 80.1% to 43.4%. This provided support for lower decline rates than reported for this species, but the fields sampled had problems with volunteer potato plants, also a common occurrence in the UK.

The Agriculture & Horticulture Development Board (AHDB) project 'Managing the decline of PCN (R269)' was completed in 2009 and aimed to identify the key factors that affect PCN decline and to explore the manipulation of fungal parasites to increase egg mortality as an alternative to the application of chemical nematicides. Findings that remain relevant in 2016 are that weak interactions between soil type and cropping regime that affected PCN decline were not sufficiently large to be used as a method of managing PCN infestations. An inverse relationship was observed between the number of eggs per cyst and the level of infection by fungi in some soils but not others and the amount of infection on a single occasion was not predictive of PCN decline rates. Soil chemical properties could not be used to predict PCN decline rates in the limited range of soils tested, which makes the relationship of PCN with soil type a complex one. Although the majority of break crops had no effect, *Solanum sisymbriifolium* significantly increased PCN hatch (but not population levels) in laboratory tests and could therefore be considered in PCN management regimes.

*3.2.6. Multiplication rates*

In any one year, PCN has the potential to increase up to 100-fold (Evans & Kerry, 2007). Trudgill et al. (2014) recorded multiplication rates of 46-100 fold with *G. pallida* with a low initial population (Pi) in untreated fields of less than 10 eggs per gram. The work was done in eight commercial potato fields, with all untreated plots being intensively sampled for PCN densities at planting (Pi) and again at harvest (final population or Pf). Regressions that form the basis of the 'PCN Calculator' displayed on the AHDB website (http://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator) were used to analyse the results, but there were major differences between sites and varieties. The results showed that the 'PCN Calculator' tended to underestimate the maximum multiplication rate and overestimate the maximum population density. Multiplication of PCN is known to be inversely density-dependent, i.e. greater at small than at large values of Pi, and hence the importance of keeping PCN levels low, but it is clear field conditions create a complex situation that is hard to predict.

*3.2.7. Influence of Temperature*

Soil temperature greatly influences hatching, mobility, infectivity and lipid (fat) utilisation of PCN juveniles (Robinson et al. 1987b). Hence it is very important in PCN management, particularly if trap cropping and pesticides at planting are used, and if estimates of the possibly detrimental effect of climate change on yield are to be relied upon. A review of the literature finds data relating to the effects of temperature on life cycle very variable, especially in field conditions, with different soil types and localised differences in temperature and different varieties all leading to different development times being recorded. Laboratory simulations appear to offer only an estimate of development, so only further studies in a variety of UK field conditions would lead to a refinement of our knowledge.

So far, studies support the view that, generally, *G. pallida* is better adapted to cooler conditions and hatches earlier than *G. rostochiensis,* whilst *G. rostochiensis* hatches more readily in warmer soils (Turner & Evans, 1998), although differences between local populations of the same species have been recorded. Ellenby & Smith (1975) found a difference in response to low temperatures (10°C) between two populations of *G. rostochiensis* in Newcastle (north-east England) and Ayrshire (south west Scotland); the latter population showed adaptation to its local cultural conditions and had a high hatching rate at 10°C than the population in Newcastle.

A nominal base development temperature (i.e. the temperature at which activity begins) of 3.9ºC for *G. pallida*, but 6.2ºC for *G. rostochiensis* was developed by Mugniery (1978). Estimates of the length of the life cycle can be made by recording daily the number of degrees over these base temperatures, upto the predicted 450 and 398 day-degrees for *G. pallida* and *G. rostochiensis* respectively, which represent a more accurate estimate than just days. Ebrahimi et al. (2014), made observations on the life cycle of both species in Belgian soils using locally popular varieties, and also concluded that *G. pallida* had a base temperature of 4°C, requiring 450 day-degrees, and that *G. rostochiensis* had a base temperature of 6°C, requiring 398 day-degrees. However, Kaczmarek et al. (2014) found base temperatures varied, estimating one of 7°C for *G. pallida*, which has implications for estimating the likelihood of second generations.

A few UK trials have started to record soil temperatures to enable better predictions for PCN development, but of course these differ between sites in the UK; an average soil temperature of 17ºC was recorded at Luffness in Scotland in 2010 and several other sites had average soil temperatures of >16ºC. This implies that two generations could be possible in about 18 weeks and, given the faster life cycle of *G. rostochiensis*, is more likely with this species. However, in another year, Luffness had an average soil temperature of 14.15°C and was cooler in the latter part of the growing season with little evidence of a second generation (AHDB Project R433, (Blok, 2015)); at Harper Adams, however, an average soil temperature of 14.85ºC was recorded that year, and there was evidence of a second generation based on the presence of juveniles and females in the roots in the latter part of the growing season. Soil type and field aspect must also affect temperatures and hence development.

With the increasing debate about climate change, and evidence that the climate of the UK overall is becoming warmer, questions are being asked about how increasing temperatures might affect the biology of PCN. The results of several studies suggest that if climate change results in increases in soil temperatures it will also result in increased rates and amounts of hatching for both species, leading to increased population levels on susceptible hosts and damage to potato crops, and hence an increase in cysts embedded around buds or 'eyes' on tubers, with the increased risk of spread that entails. Certainly evidence suggests that a dormancy period at the end of the first generation is not obligatory. If these predictions are correct, it will necessitate adjustment of all aspects of control, including the length of rotation. However, observations in Belgrade, Serbia, during a summer when soil temperatures were above 25°C actually extended the life cycle of *G. rostochiensis* by two months, suggesting that shorter life cycles encouraged by higher temperatures will have a maximum limit perhaps lower than predicted (Bacic et al. 2011). Similar studies by Munir et al. (2009) showed that the cyst and the eggs survive high temperatures in dry conditions, but under moist or irrigated conditions they lose their hatching and multiplication ability. In the UK, farmers should be encouraged to obtain data for daily soil temperatures and cyst development in individual fields that will allow a comparison with the predicted day-degrees for development, and produce an estimate of the possible risk posed by an anticipated second generation in some parts of the region.

However, climate change could be also be seen as a series of complex changes, as in 2015 temperatures have been cooler than average in some parts, with above average

rainfall. Moreover, across the UK, average soil temperatures differ significantly, so the effect of climate change will not be the same in all regions, or even within regions. In the UK, *G. rostochiensis* usually completes only one generation, although a second generation may be initiated but not completed; J2s hatch from first generation eggs, but are unable to reach the adult stage (Turner & Subbotin, 2013). However, in some conditions a partial or complete second generation has been observed within the growing season; females have been observed on the surface of tubers and "pecking" skin damage can occur which may be the result of a second generation (Blok et al. 2011). These findings need to be confirmed, and separated from infestations caused by an extended emergence.

Within the protection of the semi-permeable egg shell membrane, juvenile PCN can survive freezing to extremely low temperatures by supercooling (Wharton et al. 1993). Once hatched, the juveniles cannot survive freezing in the soil (Perry & Wharton, 1985). Snow cover, however, can insulate nematodes from the effects of freezing and thawing.

*3.2.8. Influence of soils*

Soil type may influence the life cycle of PCN, by allowing ease of movement (or not) of the juveniles through it, but also by affecting the availability and movement of hatching factors in the potato root diffusate, increasing hatch in highly organic and sandy soils, but not in clay soils (Devine & Jones, 2001). Trudgill et al. (2014), found that the yield of cv. Maris Piper (given a resistance rating of 2 against *G. pallida* and 9 against *G. rostochiensis*) was hardly affected in a highly organic soil with a Pi (initial population) of over 200 eggs per gram, whereas the yield of cv. Sante (given a resistance rating of 4 against *G.pallida* and 9 for *G. rostochiensis*) was decreased from a potential of about 60T per ha to 20T per ha in a light silt with a Pi of 20 eggs per gram of soil. There is a higher rate of hatch under host crops in sandy soils than in peats and clays (Jones, 1970). Trudgill (2001) produced data to illustrate a significant reduction in the yield of variety Maris Piper grown in sandy loam compared with peaty loam, both infested with *G. pallida*. Manorama et al. (2013), noted that soils with a lower pH and high nitrogen content recorded more PCN populations. Soils also influence the rate of decline of PCN populations. In general, the importance of soil type in the web of interactions with PCN may not be fully realised.

*3.2.9. Host plants*

A review of the known host range of PCN was made by Sullivan et al. (2007). In the UK the only cultivated host plants are potato, tomato and aubergine. Other members of the

family Solanaceae, for example bittersweet (*Solanum dulcamara*) and black nightshade (*S. nigrum*) are considered poor hosts (Anon, 1989). This was confirmed by recent studies of weed hosts in Idaho, USA (Boydston et al. 2010), but here the native hairy nightshade (*S. physalifolium*) was considered a good host. The researchers considered the eradication any nightshade species may be a prudent safeguard in controlling PCN.

*3.2.10. Local spread*

Local spread of PCN cysts is aided by dispersal mechanisms such as wind, flood and movement of machinery. Estimates of rate of spread suggest 5.3km a year radially from the site of the first detection, and 212km per year over 40 years (Banks et al. 2012). However, such measurements are of little practical benefit, as the methods of detection are only accurate at a certain level of infestation. Thus once PCN is detected in a field, one must assume it is present throughout the field, and probably on other parts of the farm, depending on the movement of machinery, for example.

*3.2.11.The increasing impact of molecular research on knowledge of PCN biology*

A draft genome sequence of *G. pallida* has been assembled, together with transcriptomic data from most of the nematode life stages, with a focus on the life cycle stages involved in root invasion and establishment of the biotrophic feeding site. The repertoires of genes likely to be important in understanding the unique biology of cyst nematodes and those that represent potential chemical targets and other targets for control have been analysed (Cotton et al. 2014). A recently assembled draft genome for the closely related species *G. rostochiensis* will also allow valuable comparative studies, and recent molecular studies are revealing a greater understanding of the biology of the PCN species, such as how PCN manages to parasitise potato roots without being ejected from them (discussed in 3.2.3.). At present there are no practical implications for PCN management.

Both PCN species show inherent genetic variation, but *G. pallida* more so than *G. rostochiensis* in Europe, due to the complexity of the original introductions from South America. In any field there might be not only a mixture of the two species, but also different genotypes, that can result in a varied field population. It is the composition and characteristics of the field population that need to be known for an effective management strategy. Current research at the James Hutton Research Institute in Dundee is investigating genetic variation in the UK, and recent analysis of DNA from individual cysts showed that the vast majority contained DNA of a single mitotype (i.e. a genetically distinct type as distin-

guished by a mitochondrial gene, cytochrome B) (Eves-van den Akker et al. 2015). However, it has also been shown that populations of *G. pallida* are frequently likely to be mixtures of different types and such detailed knowledge could lead to sophisticated methods of control being developed. Hopefully, this work could lead to a routine method for growers to identify the characteristics of their particular field population, and hence facilitate the choice of appropriate resistant varieties. However, a single cyst was found to contain a mix of mitotypes, which raises an interesting possibility of hybrid types occurring; more research is required.

Whether genetic variation often results in a significant effect on biology, or the adaptation of the pest to resistant plants, has not been investigated fully (Montarry et al. 2015). In one study it has been shown that populations in fields that are less than 50km apart show little genetic differences, but the first evidence of genetic differentiation appeared when a field was separated from others by an area free of farms (where *G. pallida* is absent or rare). Picard et al. (2005) used genetic tools to establish that, although one might expect low genetic variation in a species which itself has limited dispersal abilities, there seems to be extensive gene flow probably caused by passive dispersal of cysts by natural means of wind, running water, wild animals, and by anthropogenic means such as tillage, movement of infested tubers, etc. This is important information for the management of resistant varieties, which could be used to slow down the emergence and spread of resistance-breaking populations.

*3.2.12.Virulence and pathotypes*

It has been realised for many years that within both PCN species extensive variation in virulence occurs i.e. different populations of the same species are able to multiply to different extents on a range of different potato clones. Various pathotype schemes for PCN have been proposed to categorise this variation in virulence, with ‘pathotype’ being regarded as a group of individual nematodes with common gene(s) and differing from gene(s) or gene combinations found in other groups (the core populations that were used to define the pathotypes in the original schemes were simply field isolates from different places).

At the time these were a very useful way to assess the virulence diversity in relation to the available resistances in order to provide breeders information about the selection of sources of resistance for producing new potato varieties. However, the environment influ-

ences the extensive heterogeneity found in some populations that have subsequently been characterised, especially those of *G. pallida,* and when the pathotyping schemes were defined, the associated lack of a means for quantifying resistance/virulence has since caused problems in the use of these schemes. Consequently, most PCN populations cannot be reliably assigned to a particular pathotype (as per Kort et al. 1977 and Canto Saenz & de Scurrah, 1977), and they have been increasingly referred to as virulence groupings within pathotypes (Turner & Subbotin, 2013; Molinari et al. 2010). Thus the classifications are not very robust and should be treated with caution, given our current understanding of PCN virulence, and with respect to resistance in current potato varieties. A Table of the latest revision of pathotype groups in PCN was produced by Turner & Subbotin (2013) and is reproduced in a revised form here (Table 2).

*Table 2. Pathotype groups of potato cyst nematodes, Globodera rostochiensis and G. pallida (after Turner & Subbotin, 2013)* (in an accompanying file).

In the UK, *G. rostochiensis* Ro1 and *G. pallida* Pa1 and Pa2/3 pathotypes have been recognised, based on their reproduction on a set of differential clones derived from a range of wild *Solanum* species which all differ in their resistance to PCN. Although molecular methods can now be used in taxonomic and phylogenetic studies of both PCN and potatoes, they cannot yet be used to indicate the pathogenic relationship between the pest and its host.

So in the absence of any suitable replacement, the 1977 pathotype classifications remain in use, as, for example, in the AHDB GB-certified Potato Variety Database ((GB) PVD). The reason pathotypes are mentioned here is for consistency; in establishing the resistance of a variety it is necessary to use reference populations of PCN. The populations used by SASA in their trials are Pa3 Chavornay, Pa1 Ecosse and Ro1 Scottish; they are just three reference points from which reasonable assumptions can be made. Thus the assignment of a particular resistance score does not mean that all populations in the UK will react in a similar way. Indeed, as illustrated inTable 2, many populations do not fit into the assigned categories, showing that virulence can be very variable. Continued vigilance is needed to identify populations with unusual virulence in the field, that cause cause problems with commercial varieties.

A prime reason why the EU PCN Directive continues to restrict potato imports into Europe, is to prevent the accidental importation of any further PCN genetic diversity present in South America, including virulence that would be able to overcome the resistance currently available in European potato varieties (Hockland et al. 2012). Eves van den Akker et al. (2015) have recently demonstrated a new level of characterisation at the country scale in Scotland, using positive samples from SASA to show the distribution of introductions. This work will be expanded to include samples from England and Wales when they are available. If virulence markers are developed, then a map of the distribution of virulence can also be produced.

The threat of new genotypes being imported into Europe may not just exist in trade with the Americas; Karnkowski et al. (2011) reported an interception of *G. pallida* in Poland from Cyprus, that appeared to be more similar to South American populations than those in Europe.

## *Chapter 4. Characteristics of potato varieties*

Resistance mechanisms used by plant breeders can be complex, but are set out here for reference. However, an understanding of the effects of the resistance mechanism employed, combined with an increasing knowledge of plant-nematode interactions, can improve the success of PCN management strategies.

### *4.1 The development of resistant varieties by plant breeders*

Resistance of the host limits the development of the feeding structure of the nematode, the syncytium, thus restricting or preventing the nematode's development. The disruption of syncytium development can also affect the adult sex ratio, skewing it towards males, effectively diminishing the reproductive potential of the population.

The extent to which the plant is successful in this resistance is vitally important in a sustainable cropping system, with its minimum impact on the environment. Since potatoes were first imported into Europe the original varieties have changed as breeders sought to develop new varieties to suit each of the marketing sectors, but a commensurate resistance to PCN has proved problematic to those searching for sustainable solutions in management. The breeding of a new variety is a slow process, involving phenotypic screening of germplasm in wild diploid relatives of potato with appropriate PCN populations to identify potential resistance sources, and then determining the genetic basis of the resistance. The aim of breeding programs is to develop lines in which resistance sources are present in high dosage states in the tetraploid potato genome. These lines are then used to produce progeny from which other qualities required in commercial varieties are selected. Further crossing is done into adapted genetic backgrounds that have desirable agronomic traits such as yield and quality. The lead time for effective introgression of a new resistance and then producing a commercial variety can be 10 years or more.

The first deliberate search for resistance to PCN and the selection of PCN-resistant genotypes was initiated following declining potato yields in Europe during the 1920s and 1930s

due to shortened crop rotations in response to population demands for food. Resistance was identified in both cultivated and wild species, but development was complicated by parallel research into the identification of species and populations possessing different traits within species. Once the two PCN species were clearly identified in the 1970s, it became clear that it was essential for growers to know the individual species in their fields.

Ellenby (1945) in the UK and Mai (1951) in the USA both initiated searches for resistance to PCN, identifying resistance in both cultivated and wild species. The *H1* resistance gene from *Solanum tuberosum* ssp. *andigena* CPC1673 (Ellenby, 1952), which confers resistance against *G. rostochiensis* Ro1 and Ro4 pathotypes, was introgressed relatively quickly following its discovery in the 1950's, and given its genetic simplicity, it has been relatively easy to use in breeding programmes. It has been incorporated into many commercial varieties such as Maris Piper and growers can now choose from a good selection of *G. rostochiensis*-resistant varieties which remain fully effective after several decades of use in the UK. A molecular marker for *H1* (TG689) is now used routinely for selection of progeny with this resistance and this has greatly simplified the selection process as the nematode phenotyping assays are no longer required. Despite the amenability of *H1* for use in breeding, the locus for this gene has proven to be complex, with numerous possible candidate genes thus making the process of determining which is the *H1* gene difficult to prove. However, the durability of *H1* is attributed to it being a single major gene that is amenable to resistance breeding and the limited genetic diversity of *G. rostochiensis* that has been introduced in the UK, thus restricting the potential for this resistance to be overcome.

For *G. pallida* the situation is more complex for two main reasons; resistance that has been found in *Solanum* spp. has tended to be genetically complex and it has been difficult to introgress complex traits distributed on several chromosomes and retain high levels of resistance. Secondly, it appears that there have been several genetically distinct introductions of *G. pallida* into the UK that are phenotypically different in relation to different sources of resistance. However, significant progress in producing varieties with high levels of resistance to *G. pallida* has recently been achieved and further progress is expected using marker assisted selection (MAS) to assist with the selection of progeny from breeding programs. This is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest e.g. productivity. MAS has recently been used to combine differ-

ent quantitative trait loci (QTLs) to produce additive and broad-spectrum resistance (Dalton et al. 2003).

The development of tools to investigate genetic structures is enabling a greater understanding of resistance which can assist plant breeding programmes. Frequently, resistances to PCN are found in “hot-spots” which typically are clusters of resistance and pseudo-resistance genes. For example, Gpa, Gpa5, Grp1 are on the short arm and GroVI and *H1* on the long arm of chromosome 5. Resistance to various pathotypes of PCN has been mapped to 14 loci on 8 potato chromosomes (Gebhardt and Valkonen, 2001, Bakker et al. 2004, Finkers-Tomaczak et al. 2011) including 4 major genes (Gro1, H1, GroVI and Gpa2) and four major QTLs for partial resistance. No single gene with resistance to the dominant pathotypes of G. pallida found in the UK Pa2/3 has been identified. Quantitative resistance comprised of more than one locus usually consisting of large and smaller effect QTLs has been found in several wild species. Large effect QTLs from *S. spegazzinii* (Caromel et al. 2003, Kreike et al. 1994) and *S. vernei* (Bryan et al. 2002, van der Voort et al. 1998, 2000) have been mapped to the short arm of chromosome V, and a large effect QTL from *S. tuberosum* ssp. *andigena* CPC 2802 has been mapped to chromosome IV. QTLs from *S. tuberosum* spp. *andigena*, *S. vernei* and *S. spegazzinii* with lower effects have been mapped on chromosome IV, VI, IX and XII (Bradshaw et al. 1998, Bryan et al. 2002, Caromel et al. 2003, van der Voort et al. 2000). Other sources of resistance in potato collections such as the CPC are still under investigation.

Most UK field populations of *G. pallida* have been found to display a spectrum of virulence on resistance derived from *S. vernei* (Morag, Sante, VTN 62-33-3) or from *S. tuberosum* ssp *andigena* CPC 2802 (breeding lines 11415 and 12674) (Phillips and Trudgill, 1998). They were collectively described as pathotypes Pa2/3 as there was no clear distinction between these two pathotypes. This could be due to field populations being a mixture of virulence types (Dalton et al. 2013) and this is supported by a recent study that found that many Scottish field populations are likely to be mixtures of different introductions and therefore genetically complex (Eves-van den Akker et al. 2015). Generally the UK populations tested by Phillips and Trudgill in 1998 were also more virulent on the *S. vernei* than *S. tuberosum* ssp *andigena* CPC 2802 derived resistance, with the Luffness population being particularly virulent on the former. The results of work by Dalton et al (2013), however, differed as they found that the major *S. tuberosum* ssp *andigena* CPC 2802 QTL (GpaIV$^{s}_{adg}$) was less effective that the *S. vernei* (GpaV) QTL when tested with the Swiss *G. pall-*

*ida* population Chavornay. This discrepancy prompted an investigation into the exact source(s) of the *S. vernei* used in various breeding programs (Milbourne et al personal communication) and has led to the conclusion that the high levels of resistance found in recently developed varieties such as Innovator (HZPC) is probably attributable to the use of *S. vernei* accession LGU8 whereas *S. vernei* accession V24/20 is found in the pedigrees of Morag, Sante and VTN 62-33-3. The use of different *S. vernei* accessions by different breeding programs can have not only different consequences regarding the level of resistance achieved, but also effect whether diagnostic molecular markers are transferable between QTLs.

A further pathotype of *G. pallida*, Pa1, has been reported in Scotland and Northern Ireland and a recent report suggests that this pathotype may be more widespread than originally reported (Eves-van den Akker et al. 2015). Resistance from *S. tuberosum* spp. *andigena* and *S. vernei* also provides partial control of the Pa1 pathotype (Phillips and Trudgill, 1998). The *H2* resistance gene from *S. multidissectum* PH 1366 (Dunnett, 1962) confers high levels of control to Pa1 and partial control for Pa2/3 (Blok and Phillips, 2012). The *H2* resistance is another candidate for combining with other resistances to *G. pallida* as stacking of resistances has been shown to be effective in several studies (Caromel et al. 2005, van der Voort et al. 2000, Tan et al. 2010, 2009; Dalton et al. 2013). Stacking the two partially effective QTLs GpaV and $GpaIV^{s}_{adg}$ (using genetic markers to identify genotypes from a biparental cross possessing both loci) resulted in an additive effect that exceeded the individual resistance levels conferred by the individual QTLs independently (Dalton et al. 2013).

Plantard et al. (2008), and later Subbotin et al. (2011), analysing ITS rDNA gene sequences, showed by phylogenetic analysis that *Globodera* from the Americas were genetically different from those in Europe, Asia, Africa and Oceania. Other research has shown that the range of genetic variability of the PCN species in Europe appears to be relatively small, and breeders have developed potato varieties that are only resistant to the particular populations of *G. pallida* and *G. rostochiensis* found in Europe or even in individual countries, which makes the potato industry particularly vulnerable to the import of populations with different characteristics, especially from South America, the recently proven heartland of PCN (Hockland et al. 2012). Thus the fact that only a small number of genes is being used to confer plant resistance should be stimulating the investigation of new protective strategies. As current or new potato varieties are tested using only standard test populations, it is therefore important to record and investigate populations that seem to be

overcoming available plant resistance so that the characteristics of populations and alternative strategies can be determined. It is the characteristics of populations of PCN in a field, not of a single individual, that makes management a challenging proposition.

### *4.2. Pathogenicity, Virulence, Resistance and Tolerance: understanding their effects for PCN management*

#### *4.2.1 Definitions and Introduction*

Successful use of commercially available potato varieties requires an understanding of their influence on PCN population dynamics, as they differ greatly in the extent to which they permit PCN to multiply on them (Whitehead & Turner, (1998)). Several characteristics need to be understood, and will be defined here. Commonly used terms are pathogenicity, virulence, susceptibility, tolerance and intolerance, and resistance. The following definitions proposed for this Reference Guide are mainly based on those by Trudgill et al. (1998), but expanded here to give a fuller explanation.

*Pathogenicity* relates to the capacity of each PCN population to cause damage or yield loss of tubers. This can vary according to the species present, as well as environmental factors. The range of genetic variability in *G. pallida* in particular means that even within species there is potential for a range of pathogenicity to develop, which can be encouraged by the characteristics of the potato plant being grown. It is important that such characteristics in relation to PCN are understood, so that the consequences of using a particular variety are clear.

*Virulence*: is a measure of pathogenicity and is recorded in comparison to standard populations of known virulence. Much literature refers to named pathotypes of each PCN species, in an effort to explain the virulence of a particular population, but few field populations have yet been labelled in this way, making such an approach of little practical significance. Much preferable is to use the term ‘population’ in relation to a field or locality e.g. the Luffness population.

*Susceptible varieties*: These are good hosts for PCN. A fully susceptible variety allows the nematodes to multiply freely on roots, stolons and tubers.

*Tolerant*: This characteristic is vital to understand when choosing varieties. It may be manifested in several ways, such as early vigour and relatively large size of the haulm (Trudgill

& Phillips, 1994). Root systems tend to grow larger in heavily infested soil than in lightly infested or nematicide-treated soil, so partly compensating for the reduction in the top/root ratio (Trudgill & Cotes, 1983). Tolerant plants can yield well despite damage by PCN but hence may not show symptoms until population levels are very high, thus masking the potential problem. e.g. cvs Cara (resistance rating of 2 for *G. pallida*, and 9 for *G. rostochiensis)*, Maris Piper (resistance rating of 2 for *G.pallida* and 9 for *G. rostochiensis*) and Vales Everest (resistance rating of 6 for *G. pallida* and 4 for *G. rostochiensis*). Where there is a moderate to high level of tolerance but no resistance, the crop is able to successfully grow and develop but the nematodes will multiply, leaving a legacy of elevated levels of PCN, and the tolerance may eventually be overcome. Nematicides/nematistats may have a role in keeping PCN levels down on resistant, tolerant (and intolerant) varieties.

*Intolerant*: Some plants are very intolerant of PCN, such as varieties Pentland Dell, Maris Peer and Marfona (which all have a resistance rating of 2 for both species), Sante (which has a resistance rating of 4 for *G. pallida* and 9 for *G. rostochiensis*) and Lady Rosetta (which has a resistance rating of 2 for *G. pallida* and 9 for *G. rostochiensis*). These plants will not be able to offer any significant defence against attack by PCN, and symptoms are likely to appear at around the detection level. e.g. as a result of analysing 500g-600g soil taken from a hectare. As well as the common PCN effect of a reduction in top/root weight ratio, intolerant varieties also show a reduction in the weight and length of their root systems when grown in heavily infested soil (Trudgill & Cotes, 1983). Intolerant varieties are estimated to have a low damage threshold population of approximately 2 eggs per gram of soil (Whitehead & Turner, 1998).

Determining the extent of tolerance of different varieties is best performed in the field and requires several uniformly-infested sites so that environmental effects can also be taken into account. As well as being characteristic of a variety, tolerance is also affected by the availability of crop nutrients and water as well as the PCN population. Two AHDB projects sought to develop a method for assessing tolerance and generate more data for growers in relation to *G. pallida* (Keer, 2013, 2007) but the work showed that performance varied from site to site, due at least in part to the different environmental conditions between the three years' trials. Thus it has not yet been possible to quantify tolerance in any meaningful way, or to assign varieties into different tolerance classes. Farmers and advisers are encouraged to contact breeders for more advice, or assess data from their own fields.

*Resistant*: Host resistance to PCN is defined as the ability of the host to restrict the number of developing females (Trudgill, 1991) and thus the reproductive potential of the pest, to varying degrees. This ability is recognised as not being guaranteed against all individuals in a population. It is usually attributed to specific genes, or to wild species that were the source of the resistance. With high levels of resistance, such as those provided by the *H1* gene, multiplication of *G. rostochiensis* is minimal (Gebhardt et al. 1993 and Paal et al. 2004). However, with varieties showing low levels of resistance, some multiplication can occur, and could lead to a shift towards populations that are more virulent than the initial population (Turner & Fleming, 2002(a), 2002(b); Phillips & Blok, 2008; Fournet et al. 2013).

*4.3. Categorisation of resistance levels*

Varieties that allow significantly less multiplication than fully susceptible ones have been termed partially-resistant, but now, under the recent EU Council Directive 2007/33/EC (Potato Control Directive, (Anon, 2007)), a standardised method for testing and scoring potato varieties for resistance to PCN has been accepted by EPPO and EU Member States to bring clarity to the subject, and to streamline the various resistance ratings across Europe. This standard was published by EPPO and allows a quantification of resistance to be made (Anon, 2006). The scores can be incorporated into models of the population dynamics of PCN. All varieties are now scored on a 1-9 scale, based on pot tests that assess the relative multiplication rates of European 'reference populations' of PCN on the candidate varieties. In the UK these are *G. pallida* Pa1, Pa2/3 and *G. rostochiensis* Ro1; in the first year all candidate varieties are tested against Ro1 and Pa3 reference populations. If resistance (defined as the relative susceptibility of less than than 50%) is found, then tests are repeated in a second year and Pa1 is then included as an add-on 1 year test for varieties showing resistance to Pa3. Typically, when the Pa1 and Pa3 populations are used, similar results are achieved, albeit that most varieties score 1-2 points higher against the Pa1 population than the Pa3 population. However, practically, the resistance rating should be viewed as an overall one for *G. pallida*. The scoring system is based on 'relative susceptibility' which is calculated by expressing the PCN population produced on a standard susceptible variety e.g. Desiree. For example, if a variety limits PCN multiplication to 50% of that on Desiree, it will have a resistance score of 3. If the final PCN multiplication is 25% of that on Desiree, it will score 4, and so on. To illustrate this, the variety Maris Piper, which has a resistance score of 9 to *G. rostochiensis* Ro1 limits PCN multiplication to less than 1% when compared with Desiree. However, Maris Piper is

susceptible to *G. pallida* populations of pathothypes Pa1 and Pa2/3 and has a score of 2. The variety Vales Everest has a resistance score of 6 to *G. pallida* Pa2/3 and limits multiplication to between 5% and 10% of that on a susceptible variety; it has a resistance rating of 4 to G. rostochiensis Ro1. with '1' representing plants with the lowest resistance and '9' representing plants with the highest resistance. Annual evaluation tests are done at SASA (Edinburgh) (http://www.sasa.gov.uk/seed-ware-potatoes/nematology/pcn-resistance-testing) and NIAB (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/439430/vcu-procedure-potato15.pdf) (at Cambridge, *G. pallida* only) and results are added to the UK National List and to other characteristics provided in the (GB) PVD (http://varieties.ahdb.org.uk).

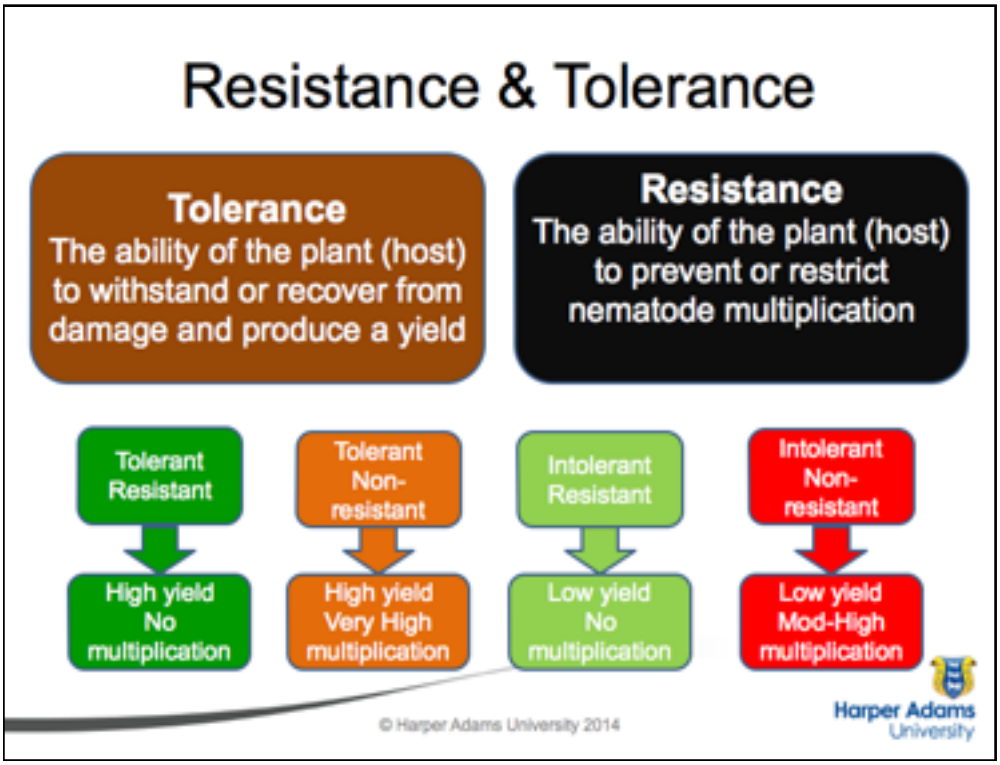
Some varieties grown in the UK have undergone testing in another EU country. The PCN resistance ratings for these varieties, such as Innovator, which has shown a high level of resistance to *G. pallida*, can be found in The European Cultivated Potato Database (ECPD) (http://www.europotato.org/menu.php). Unlike the (GB) PVD, the varieties listed have not undergone similar testing and it is in reality a catalogue of claims in relation to varieties. However, it is the result of collaboration between participants in eight European Union countries and five East European Countries and is intended to be a source of information on varieties maintained by them. Within the database, all observations are attributed to their source. Although the varieties in the (GB) PVD and the ECPD have undergone different tests before they are included, the AHDB and SASA are working to make the (GB) PVD far more comprehensive in relation to varieties that have not undergone the independent variety trials (IVT) (http://potatoes.ahdb.org.uk/publications/r447-independent-variety-trials-2011-2014). Thus it will eventually provide scrutinised information on any variety grown in GB. The intention is to make clear the source of the information, whether it is from breeders' claims or IVT, so that visitors to the site can make informed decisions on different varieties and their level of resistance.

The use of previous terms such as 'partial' and 'full' resistance are now considered obsolete and the level of resistance should now be expressed by referring to the new European scale, so all users understand the degree of resistance being offered. Likewise, varieties should rarely be described as totally resistant; any field population may exhibit a wide genetic variation, and may also have the two species of PCN which thus requires careful monitoring. There will always be a high risk that one individual PCN in any field population will have the genetic capacity to overcome a plant's resistance. If the same variety is

grown season after season, such individuals will be selected over time and become the dominant type, assuming that other environmental factors do not influence its fitness.

We have defined tolerance and intolerance, and resistant varieties will exhibit one of these characteristics, which will affect both the potential yield and the resident PCN population. For example, whilst a resistant variety will prevent or restrict PCN multiplication, according to it's rating, it will produce a higher yield if it is tolerant, but a lower yield if it is intolerant. Likewise, a susceptible but tolerant variety will result in a high yield but also a very high multiplication rate, whilst a susceptible but intolerant variety will result in a low yield and also a moderate to high multiplication rate of PCN. It is thus important that when resistant variety is chosen, that the tolerance is also considered. However, as has been stated previously, it has proved difficult to rate tolerance for many varieties in field situations, so the actual effect of a particular chosen variety on yield and the residing PCN population in any particular field needs to be monitored. Fig. 8. shows a summary of the distinctions between tolerance and resistance.

*Fig. 8. The consequences of using potato varieties with different combinations of resistance and tolerance (copyright Harper Adams University 2014).*




*4.4. Effect of using resistant varieties in the UK and Europe*

Plant resistance offers an environmentally friendly and sustainable method of PCN management, and cultivating varieties with high levels of resistance is the most highly effective control measure for suppressing PCN multiplication. However, it is the continued use of potato varieties resistant to *Globodera rostochiensis* (e.g. Maris Piper and Cara, both with the highest resistance rating of 9 to this species, but only a rating of 2 against *G. pallida*), and the much more infrequent cultivation of varieties with any significant resistance to *G. pallida*, that has led to the increasing importance of the latter species. Data from soil tests from land intended for use for seed potato production in Scotland show this effect quite clearly (Figs. 9 and 10).

*Fig. 9. Incidence of G. rostochiensis in land intended for use as potato seed production in Scotland (reproduced with kind permission of SASA).*

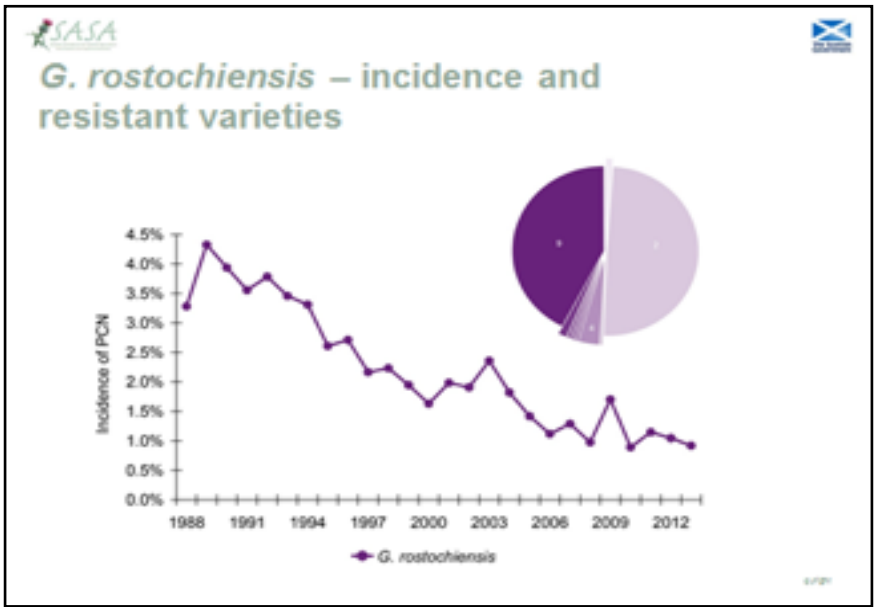
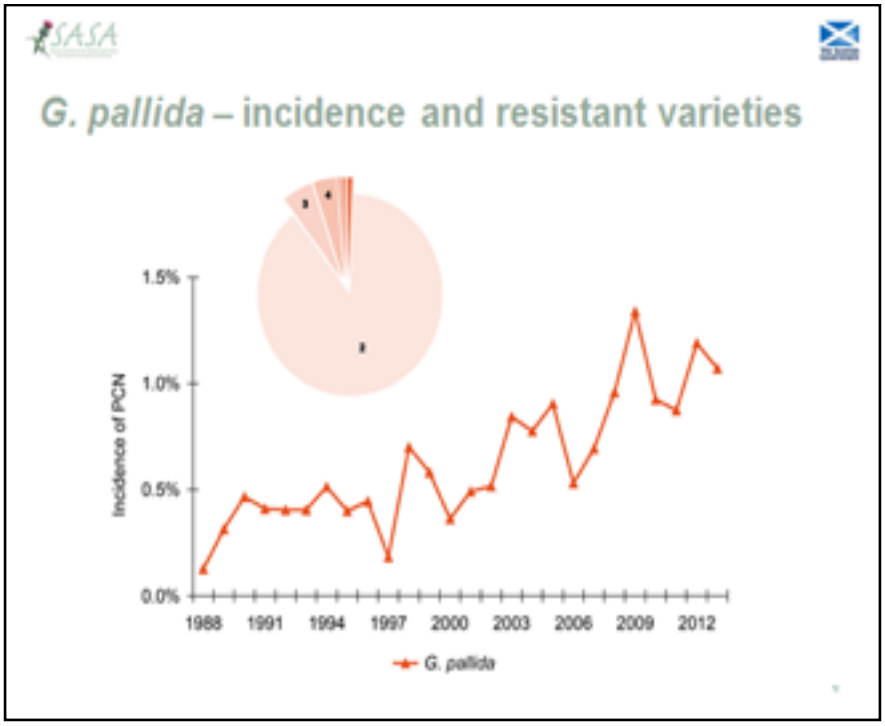



*Fig 10. Incidence of G. pallida in land intended for use as potato seed production in Scotland (reproduced with kind permission of SASA).*




When a potato variety is classified in the middle of the resistance scale, then it is more likely that field populations of PCN may increase or decrease on that variety, depending on the influence of other environmental factors, or the presence of a mixture of PCN species. For example, Trudgill et al. (2014) found that variety Sante (described in the (GB) PVD as having a resistance level of '4' for *G. pallida* and a resistance level of 9 for *G. rostochiensis*), actually worked well against *G. pallida,* decreasing the multiplication rate.

The first varieties with PCN resistance carried the *H1* gene, derived from *Solanum tuberosum* ssp. *andigena,* effective only against Ro1 and Ro4 designated pathotypes of *G. rostochiensis*, but these were recognised then as the most common types of PCN present in the UK (Bakker et al. 2004). Their use can decrease population densities of *G. rostochiensis* by 80% or more.

Plant resistance to *G. pallida* has been under intensive study for many years. Breeders have, until very recently, only been able to produce varieties that offer a low level of resistance to *G. pallida,* such as Santé (rated 4), Nadine, and Valor (both rated 3), along with full resistance to *G. rostochiensis* (rated 9 in all cases). This is because the resistance, from the wild potato species *Solanum vernei*, is based not on a single major gene, but on several genes, which result in the production of a less active root diffusate and thus results in the invasion of fewer larvae, of which fewer still develop past the invasive stage (Dale & de Scurrah, 1998). This means that the plants reduce, but do not prevent, the multiplication of *G. pallida* (Lane & Trudgill, 1999). *S. vernei*-derived resistance exhibits continuous

variation for PCN resistance, supporting the view that this source of resistance is largely inherited in a polygenic manner; today there are two sources of such resistance being studied, and one seems much stronger and robust than the other (Vivian Blok, personal communication).

The *Gpa2* gene, used more recently, is only effective against specific populations of *G. pallida (*van der Vossen et al. 2000). In the UK there are localised areas where virulent populations of *G. pallida* have been found*,* e.g. Luffness in Scotland, but generally the poor uptake of varieties resistant to *G. pallida* makes assessment difficult. There is evidence from Europe, where so-called totally resistant varieties are grown more widely, of an increase in virulent types. In Germany, for example, there have been recent reports of a new highly virulent population of *G. pallida* in their starch production area. The early indications are that this population can overcome the resistance of varieties showing high resistance to *G. pallida*. It is thought that this pathotype has been selected through the intensive cultivation of resistant varieties (Giltrap, 2015).

Thus if resistant varieties are to be grown more widely, potential strategies need to be developed to ensure the durability of such gene resistance in the field; whilst the *H1* gene for resistance to *G. rostochiensis* has proved remarkably durable, there is little evidence concerning resistance to *G. pallida* because such varieties have not been widely grown to date. The forces affecting the emergence of virulent populations are complex, multi-factorial and involve both the host and the parasite of the plant-nematode interaction. It has been suggested that alternating varieties with a range of resistance against *G. pallida* will probably slow the selection of virulent nematode populations in the field, especially given the scarcity with which such varieties are grown. This theory was tested by Beniers et al. (1995), who found that this was not true when varieties contained closely related resistance genes. However, when varieties containing resistance genes from widely separated sources were alternated, the rate of selection of virulence was retarded considerably. Thus the industry needs more long-term research to test ways of capitalising on the resistance found in national potato varieties. A review of potential strategies to improve the durability of the resistant genes has recently been published, but it is clear that further research is required (Davies & Elling, 2015).

In some countries, field populations of PCN have been collected and tested against resistant varieties (Limantseva et al. 2014). In the UK, however, there are few published obser-

vations on the effect of continued use of varieties with a range of resistance against *G. pallida*. Phillips & Blok (2008) observed four field populations of *G. pallida* grown on four potato genotypes with a range of resistance for 12 generations. The resistance was derived from either *Solanum vernei* or from *S. tuberosum spp. andigena* CPC2802. After 12 generations, selection pressure was shown to have increased reproductive ability, but increases were specific to the source used. The average increase on the ex *S. vernei* clones was from 11% reproduction by the unselected populations to 35.5% reproduction after selection. On the clones derived from CPC2802, which had higher levels of resistance, the increases were larger with an average of 6.6% reproduction for the unselected but 47.4% reproduction after selection. The response to selection differed amongst the initial field populations with some rates of reproduction increased by as much as 79%. A RAPD-based analysis of the original and sub-populations after selection indicated small but consistent changes in the genetic structure, which could have been the result of the selection pressure per se and/or the bottlenecks that the population had gone through.

In The Netherlands, there is a long-term project to determine the long-term effect of planting potato varieties with low levels of resistance on population densities of *G. pallida* (Been et al. 2014). In the first 4 years there was no decline of the population, but after 10 years there was a moderate decrease. The decline was greater than natural decline without cropping combined with soil fumigation. There was a very significant decline of 77% in one non-cropping year. One or two fields seemed to have higher populations under the cropping regime than the others, and a higher virulence was suspected. After 20 years there has been a decline of PCN in all fields.

*G. pallida* populations are capable of adapting to the resistance of potato genotypes carrying the QTL GpaV gene from *Solanum vernei*, but this does not necessarily allow the adaptation to other resistance sources (Grenier et al. 2014). Work by Fournet et al. (2013) investigated the indirect consequences of adaptation on the durability of resistance in different potato genotypes harbouring the same resistance QTL. They studied *G. pallida* populations in a long term trial, and found that the nematode populations were able to adapt to the resistance of four potato genotypes carrying the QTL GpaV from *Solanum vernei*, and that the plant genetic background has an impact upon the durability of resistance. The pattern of local adaptation observed here indicates that selection of individuals more fitted to survive in such regimes has occurred during the experimental evolution performed from the same initial nematode population, and revealed a trade-off between the

adaptation to a resistant potato genotype and the adaptation to another resistant genotype differing in its genetic background. In terms of cross-virulence between potato genotypes derived from different resistance sources (*S. sparsipilium* and *S. spegazzinii*) this study showed that the adaptation to resistance QTL GpaVvrn does not necessarily allow the adaptation to collinear GpaV loci. Such work is useful for predicting evolution of nematode populations in potato cropping and identifying durable strategies for resistance deployment.

There is no data from the UK that would assist the development of a detailed strategy utilising varieties of varying resistance, based on the variety of sources of resistance underpinning the *G. pallida* varieties with high levels of resistance currently used in the UK.

There has been research into the genetics of PCN populations, particularly *G. pallida*, and the biology of populations, in an effort to estimate the long-term effect of plant resistance in the field, but further work is needed before research can be utilised in practice (Montarry et al. 2015). If populations of either species increase despite the use of resistant varieties, then this should not be ignored but investigated with specialists. It should be remembered that plants showing any level of resistance to PCN are unlikely to prevent infestation by other nematode species such as stubby-root or root-lesion nematodes.

***Chapter 5. Yield loss; evidence for effect on yield***

The time taken for PCN damage to appear in crops depends on the frequency with which potatoes are grown, but damage usually occurs within 5 or 6 crops after the date of introduction, or about 20 years when potatoes are grown on 4-year rotations (Evans, 1979; Evans & Brodie, 1980). The greatest potential for crop losses occur in ware potato crops grown in short rotations of 6 years or less. Soil types can also influence the success of PCN and hence yield; peat or other soils with a high percentage of organic matter will support higher yields (M. Back, personal communication).

A major component in yield reduction caused by PCN is reduced vigour of the top growth and thereby a reduction in total light intercepted (Trudgill & Phillips, 1994). As a response to invasion the host plant produces many lateral roots, especially near the soil surface, and fresh laterals are produced early in the season. *G. rostochiensis* causes infested plants to become yellow and stunted, and root systems are reduced, abnormally branched and brownish in colour. Symptoms in the field first appear in small patches, which result in more subtle effects of infestation, in (i) slowing the rate of leaf expansion, and hence the time taken for the leaves of infested plants to touch which decreases the efficiency of light interception, and (ii) affecting the potential of dry matter production that is partitioned into harvestable yield. In potato, variations in the tuber dry matter and tuber size further modify yield and hence the value of the harvested crop (Trudgill et al. 1998). At low densities tuber sizes are reduced, whereas at higher densities both the number and size of tubers can be reduced. At 8 and 64 eggs/g of soil, yield losses of about 20% and 70% respectively

can be expected. *G. pallida* causes similar symptoms but is considered the more serious pest, with a quoted damage threshold as low as 1-2 eggs per gram of soil.

Seinhorst (1982) and Elston et al. (1991) proposed models for *G. pallida* that described the relationships of PCN population densities before planting with potato yield and post-harvest nematode populations. However, the damaging effects of PCN are not only determined by nematode density, but also by factors such as variety (whether it is tolerant of PCN invasion, or its degree of resistance to the prevailing field population), crop husbandry and environmental conditions, such as soil type and climate. There is a lack of UK yield data concerning losses due wholly to PCN for current commercial varieties. Whitehead (1988) quoted maximum yield losses of 17.6 t/ha with 20 eggs/g, and losses of 22 t/ha with 0-160 eggs/g, but it should be noted that a given level of infestation is not the only factor involved in estimating yield loss. A general figure of 9% to 10% for the UK potato crop has been given in numerous papers, but it is believed that this comprises data from a report by Mary Hancock of ADAS in 1988 which estimated losses of 6%, assuming no corrective action was taken, plus the then cost of nematicide application, cost of producing new varieties and cost of advisory work (Sue Hockland and Ken Evans, personal communication). PCN are said to be responsible for annual potato tuber losses of up to 9% in Europe (Turner & Subbotin, 2013).

In west Serbia, the effect of *G. rostochiensis* on 15 varieties, including both resistant and susceptible varieties, was investigated. Here the lowest yields were found on susceptible varieties such as Romano (15.2T per ha) and the highest yields found on resistant varieties such as Naviga (44.8T per ha). However, it is not known whether these yields were the consequence of tolerance (which does not necessarily have an effect on the PCN population), or the resistance mechanism.

In Australia the economic impact of PCN that spread from a small initial incursion was assessed, and models linking spread, population growth and economic impact were combined to estimate costs of spread without restriction (Hodda & Cook, 2009). The mean annual costs associated with the spread of PCN would increase rapidly initially, associated with increased testing. Costs would then increase more slowly, to peak at over AUD$20 million per year approximated to 10 years into the future. Cumulative losses to Australian agriculture over 20 years might exceed AUD$370 million without action to prevent spread of PCN and entry to new areas would occur.

PCN, as with other plant-parasitic nematodes, may also interact with other pathogens to further reduce yields. Interactions between *G. pallida* and *Verticillium dahliae* have been investigated under laboratory conditions by Storey & Evans (1987). In the potato cv. Pentland Javelin, pre-inoculation of *G. pallida* juveniles resulted in greater fungal colonisation by *V. dahliae* than when the fungus was inoculated alone. The authors suggest that the defences of the cortical cells are breached by the invasion tracts of the nematode which enable the fungus unimpeded access to the stele. However, in varieties Maris Peer and Maris Anchor, *G. pallida* juveniles were observed to induce extensive lignification of root cells (hypersensitive response) which presented a physical barrier to *V. dahliae*.

Back et al. (2006) showed a clear relationship between the density of *G. rostochiensis* present in potato roots and the incidence of stolons infected by *Rhizoctonia solani,* six weeks after planting, in field conditions. It has also been suggested that plant-parasitic nematodes *per se* also contribute to the incidence of potato diseases. Laboratory experiments in the UK tested the hypothesis that invasion and damage caused to potato roots by *G. rostochiensis* might result in quantitative or qualitative changes in the release of root exudates to subsequently affect the growth of *R. solani* in the potato rhizosphere. They showed that plant roots infested with J2s and J3s increased the growth and rate of growth of the fungus, possibly due to higher levels of sucrose in the root exudates (Back et al. 2010). Bhattarai et al. (2010) investigated interactions between field populations of the potato cyst nematode *G. pallida* and *Rhizoctonia solani* diseases of potatoes under controlled environment and glasshouse conditions, which revealed that a combination of a fast-hatching population with *R. solani* caused significantly more *R. solani* disease incidence and severity than a population of *G. pallida* with slower hatching characteristics. Bhattarai et al. (2009) had performed field experiments that showed a clear positive relationship between densities of nematodes within the potato roots and the incidence of infected stolons, stolon pruning and stem canker. Recent research in Iran on varieties Sante and Marfona also recorded that high densities of PCN increased the severity of the disease, but that effects were different between the varieties.

In addition to the damaging disease complexes discussed above, PCN infestations may influence the susceptibility of potatoes to infestations of peach potato aphids (*Myzus persicae*) as reported recently by Hoysted et al. (2015). Preliminary data suggests that nema-

tode occupation is causing a reduction of anti-herbivory feeding deterrents and terpenes. Thus the management of PCN will also have an effect on the severity of other pathogens.

The effect of PCN on 'early' potato crops tends to be less as they are lifted before many of the nematodes have had time to complete a new generation (Anon, 1989).

## *Chapter 6. Steps to determine appropriate control measures*

The management of PCN will differ from farm to farm and maybe even from field to field, depending on a range of environmental factors and management strategies. The identification of the PCN species infesting any potato field is of fundamental importance in its management. If *G. pallida* is present in mixed populations, management strategies should give priority to this species, but the situation should be monitored.

### *6.1. Introduction*

This Reference Guide provides a consensus of advice on key areas to be considered for PCN management plans. In addition to specialist opinion, information from all available publication resources and reports of R&D projects on PCN sponsored by Defra and the AHDB have been included where applicable. However, many projects which could influence PCN management strategies are on-going or currently being contemplated (see Chapter 9, Concurrent Projects and Future Research Needs).

Some chemical companies provide advice on PCN management, and should certainly be consulted when using chemical products.

A programme for control of PCN developed for Dutch growers, NemaDecide, is available on-line for individuals at the sterling equivalent (August 2015) of £2305 in the first year, followed by an annual fee of £709 (http://www.nemadecide.com/en/index.html) (Been et al. 2007). However, this system is not tailored to UK conditions. The decision support system

was developed with the aim of keeping nematodes at low, economically acceptable density levels, by using the results of 50 years of Dutch research in a new software package that would enable growers to estimate risks of yield losses, to determine population development, to estimate the probability of detection of nematode foci by soil sampling and to calculate the cost/benefit of control measures. Gaps in knowledge were given higher priority in research programmes, which are now better focused on practical problems (Been et al. 2005).

### *6.2. Sampling and Detection*

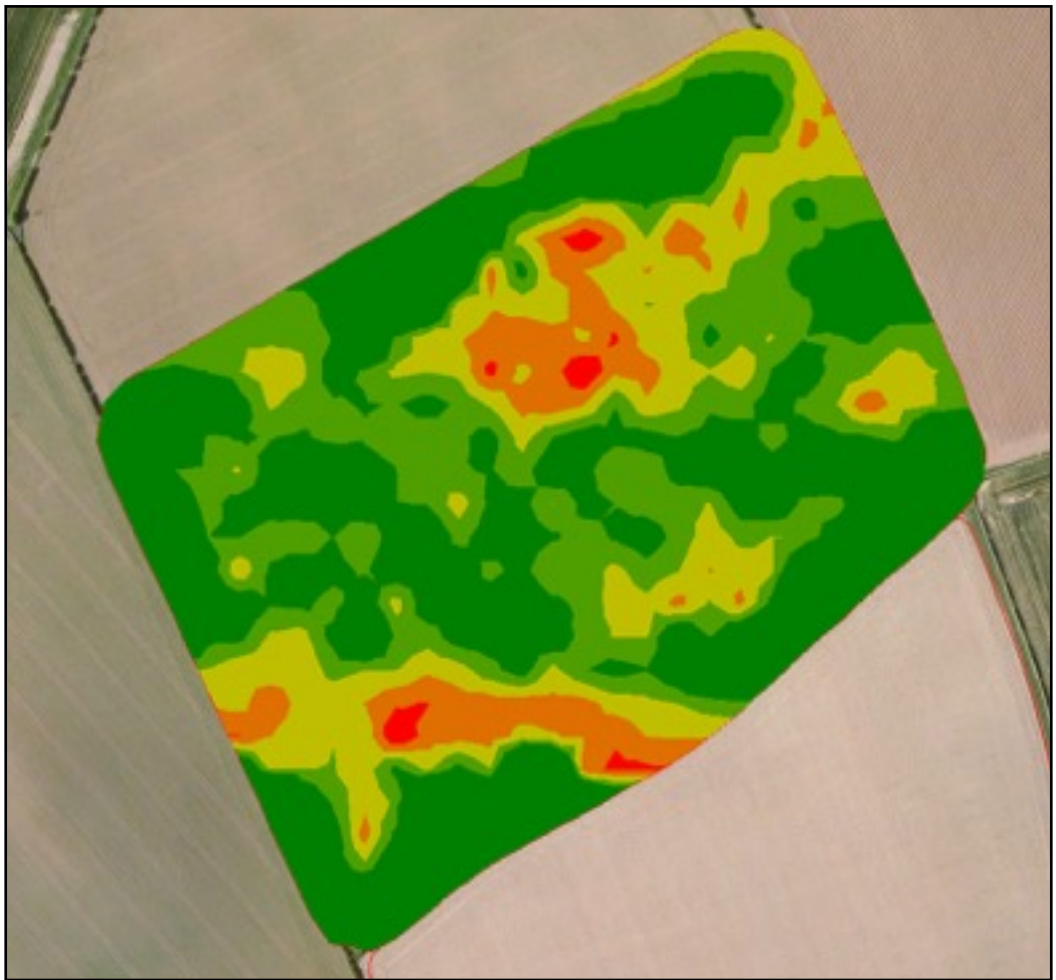
Sampling and analysis of soil samples to detect the presence and population level of PCN provide the basis for any PCN management programme. Even if PCN is not detected on agricultural land, such a result should be understood to mean just that, that PCN has not been detected, but a management plan to prevent PCN reaching detectable and hence damaging levels should be developed.
Ideally sampling should be done at harvest, under the riddle, or immediately after harvest, when PCN is at its most accessible.
Processing of samples should be done at a laboratories with accreditation for processing soil samples for PCN and subsequent identification to species.
Frequency of sampling depends on the use of the data; for the detection of PCN on uninfested land, or to monitor the levels of PCN over time. Annual sampling will provide an indication of the decline rate of PCN, whilst sampling before and after harvest will help to assess the influence of the potato varieties being grown. Remember, it is essential to manage PCN at low levels.

The AHDB have commissioned a PCN sampling project that is due to report at the end of January 2016. Thus in this Reference Guide only general advice on sampling will be presented.

The detection and correct identification of PCN remains as important as ever, to harness aspects of biology and life cycle for control. The distribution of PCN across any field is not

uniform, neither is it random. Following an initial infestation at one or more points in the field, the infestation, or focus, spreads in a patchy manner, giving rise to secondary foci, which themselves spread similarly. These small foci may appear to be isolated, but by the time the infestation is readily detectable the whole field may be infested, although the infestation may be below detection level in certain parts of the field (Southey, 1974). It may take many years from the initial infestation of a field before patches of damaged plants are first noticed (Haydock & Perry, 1998). Foci are often elliptical in shape, with the largest population density in the centre of the patch. The distribution and shape of the foci are determined mainly by mechanical operations, such as planting and harvesting, which move the soil predominantly in the direction of tillage (Been & Schomaker, 1996). The recent innovation of digital mapping, using drones (unmanned aerial vehicle or UAVs) to measure factors such as canopy development, shows potential as a tool to assist in locating areas outside the sampling 'trail' that may unknowingly be suffering from PCN infestation or other factors (Knottenbelt, 2015). Fig. 11 shows an example of a crop cover/crop vigour map from a potato field which, in those cases not suffering from other pathogens or disorders, may also serve to illustrate PCN 'hot spots' in a field.

*Fig. 11. A potato crop canopy/crop vigour map produced by digital mapping using UAVs (Courtesy URSULA Agriculture).*



The vertical distribution of PCN can vary considerably, depending on the time since the last potato harvest and subsequent cultivation operations. However, if detection is the prime objective, it has been surmised that the depth to which samples are taken within the top 20cm of field soils would have little or no effect on the result (Boag & Neilson, 1994).

PCN cysts may be first detected directly on plant roots during the summer months. However, this is usually when damage has been noticed in the field. In order to develop a management plan for PCN, a soil sampling plan needs to be developed, which ideally should detect PCN before damage has been noticed. Growers need to consider the aim of any soil sampling done, as the laboratory process for each is different. For instance:

- to detect the presence and species of PCN to formulate a management plan. A detailed analysis of eggs per gram is not necessary to determine a threshold for action - the action threshold recommended here is detection.
- to make an assessment of the effect of the PCN management plan, such as the use of resistant varieties; this would require samples to be taken before planting (Pi) and immediately after harvest (Pf), and an estimate of population levels as eggs/gram.

Soil sampling should be frequent and routine in any potato rotation, ideally before planting and especially after harvest. This is to ensure the best chance of controlling the pest at low levels, and because infestations usually occur in ‘hot’ spots which can be easily missed by most sampling plans with a potential pest distribution as illustrated in Fig. 11 above. When cultivation has taken place cysts can be found at varying depths, so timing should be borne in mind when considering a sampling regime.
An alternative method is to take samples of soil residues from under the riddle at harvest. The latter has been a long-standing recommendation in the UK, and recent field work in Belgium has provided data to show this provides a useful indicator as to levels of infestation immediately after harvest, especially if the samples are linked to particular areas in the field which enables hot spots to be identified (Goeminne et al. 2015).

Whilst potato growers should develop a management plan for PCN even if it is not at a detectable level, detection is usually the key factor which triggers action, whether the results are expressed as cysts or eggs/gram. A sampling regime can be very intensive and involve numerous cores of soil taken from a grid pattern, in order to get an impression of the distribution of PCN across a field, or, if PCN is known to be present, can involve samples

from only from known infested areas to confirm viability, or used more frequently to determine initial population densities (Pi), or final densities after harvest (Pf).

There is not a uniform procedure in the UK; different methods of sampling have developed over the years, but in general, populations below 500,000 cysts per hectare cannot be reliably detected (Sharma et al. 2014). The EU PCN Control Directive 2007/33/EC, published in 2010, introduced a harmonised sampling procedure for both seed and ware potato fields in member countries. The aim was to spearhead a more collaborative approach for the management of this pest in Europe (Hockland, 2010). It is a compromise product of much research and debate, and adopting the standard statutory rate of 1500ml/ha involves taking more soil than was previously required for statutory pre-crop sampling in all major seed potato growing Member States of the EU. A reduced rate of 400ml/ha is permitted for fields considered to be of lower risk, i.e. where previous soil testing has not revealed PCN to be present or if potatoes are being grown on rotations of 1 year in 7, or longer. These two sampling rates were established for the EU using field distributions described by Been & Schomaker (2000). The detection level of PCN should be clearly below the level at which symptoms of PCN become visible. This would be the result of a focus density with a central population density of 100 cysts/kg soil. At this focus density, an average of three secondary foci will also exist (Been & Schomaker, 1998). These secondary foci are smaller in size and were defined in the calculation as having a central population density of 50 cysts/kg soil. Assuming these foci are all present in a 1 ha unit, the population of PCN cysts in the top 20cm of soil is 4.1 million. The probability of detecting PCN with a sample consisting of 100 cores taken on a rectangular grid pattern covering the entire hectare is 90% for the standard rate and 50% for the reduced rate. To satisfy the requirements of the EU Directive, all of the sample drawn must be analysed for the presence of PCN.

As the new Directive introduced higher sampling rates, the area of land recorded as infested with PCN in Scotland has increased considerably since 2010. This is a consequence of a change in the sensitivity of the detection process, rather than deterioration in the health status of the land. The UK annual ware survey involves sampling at the reduced rate rather than the standard statutory rate. If the presence of PCN is the key to PCN management, then the statutory sampling method should be considered.

EPPO Standards for processing, detection and identification have been published (Anon, 2013a, 2013b), and have set the standard for processing laboratories in Europe. However, they have not yet been universally adopted in the UK, and there is no national accreditation scheme for such assessments. Thus it is likely that results between laboratories can vary greatly, as has been found with proficiency tests done internationally. Reasons for that might be differences in the custom-made equipment used, laboratory-specific adaptations of the method, or different experience of the operators. A detailed overview of extraction methods for cysts in soil, including advantages and disadvantages, was included in a recent EPPO Diagnostic Standard for the extraction of nematodes from soil and plant material (Anon, 2013a). In The Netherlands, a statistical analysis of nematode counts from a series of inter-laboratory proficiency tests on PCN showed there was both intra- and inter-laboratory variance that could affect management decisions (van den Berg et al. 2014). Proficiency testing procedures have been developed at Fera Science Limited (Reynolds & Owen, 2010).

Just as different laboratories use different processing methods, so each farmer will have a different requirement from the processing laboratory, and it is important that there is an understanding of both the limitations and advantages of the processes being offered. Currently an AHDB Project is underway to improve the quality and licensing of laboratory procedures in line with European standards for PCN extraction and identification that already exist (Anon, 2013a; Anon 2013b). It is important that a specialised service using recognised standards for PCN extraction and identification is used, especially as it is estimated that 25% of infested fields in England and Wales contain a mixture of the two PCN species (Minnis et al. 2002). Thus the ability of the identification method to identify and quantify both species should also be considered.

For the purposes of this Reference Guide, a few recommendations on detection are made:

1. If PCN has not previously been detected, or if the mere presence of PCN is likely to be important, such as in a seed potato crop, then the whole of the sample taken should be processed.

2. Whilst it has not been demonstrated that the extraction equipment has any effect on the detection rate, it is important that the processing equipment is of a recognised and continuing standard if population levels are being monitored.

3. Laboratory personnel need to distinguish PCN cysts from cysts of other nematode species, such as sugar beet cyst nematode, brassica cyst nematode and cereal cyst nematode.

4. The reliable identification of *Globodera* to species level depends on specialist expertise and is best achieved by using a combination of morphological and molecular or biochemical methods. This combination of skills is especially important when samples are processed from fields known to contain a mixture of PCN species, as some molecular methods may not be able to distinguish them if one species is significantly in the minority. In addition, molecular procedures need to be able to distinguish PCN from other species of cyst nematodes, as well as the native *G. achilleae* which does not feed on potato. Indeed, Mandani et al. (2010) and Grenier et al. (2010) both urge caution with the sole use of molecular tools for identification purposes i.e. more research is certainly required to ensure the target species are identified. Nevertheless, the increasing use and reliability of molecular tools, used in conjunction with morphological methods of identification, do provide an opportunity for the routine, reliable identification of PCN and other *Globodera* species (Alemu, 2014). There is no doubt that the increased use of molecular tools has and will continue to improve identification procedures, as morphological methods of identification are difficult and require specialist time and effort that is not always available during times of peak sample processing. Mitochondrial genes, the internal transcribed spacer region (ITS1-5.8S-ITS2) of the rRNA gene and D2-D3 expansion segments of the 28S rRNA gene have been studied to identify *Globodera* species (Blok et al. 2012; Mandani et al. 2010) and form the basis of many current identification procedures.

5. Novel methods which combine detection and identification of PCN in soil samples are available in some laboratories; they are not routinely available and are still being refined.

Once identified to species, further analyses may be warranted, depending on need. For example, viability assessments, and/or eggs/g, if detailed monitoring is being done to assess the effect of particular management tools, such as resistant/tolerant varieties.

*6.3. Viability*

Viability is defined here as the detection of live juveniles deemed capable of infesting a potato plant. In the UK and elsewhere, management of PCN has been related to nematode population densities estimated as eggs per gram of soil, and in general the actual number of viable eggs or juveniles has not been calculated. In regularly cropped fields, the risk associated with finding cysts in a potato rotation is not going to be changed significantly by any detailed measure of viability. Viability is important for seed producers, where the finding of a single viable cyst will result in the potential crop being abandoned, and for detailed monitoring to assess the influence of management strategies. Viability tests per se remain relatively expensive and resources should be concentrated on speciation and monitoring.

### *6.4. Current use of Thresholds for action*

> The general increase in population levels of PCN, especially *G. pallida*, and subsequent risk posed to the potential of UK potato crops, means that a concerted effort to reduce populations is now required wherever PCN is detected, or at the very least, when it is at low levels. This strategy is supported by the available scientific evidence, that PCN has the ability to multiply from population levels that are not detected to highly damaging levels in one susceptible crop. To a large extent this makes the use of detailed thresholds redundant, and resources should be invested into the identification and monitoring of the species present in a field to improve choices in management strategies.

Research into thresholds for action, based on pre-planting sample results, has been done in the UK, Europe and worldwide. However, much of this information, and its use in computerised advisory systems, has not been fully assessed and in most cases more data is required to establish their reliability. The inclusion of data from national trials on PCN, for example, needs to be considered.

Many advisory thresholds, commonly expressed as eggs per gram, are loosely based on ADAS advice given since the 1970s. As far as is known, this advice was not based on published research, but was a consensus by a group of very experienced field advisers (Hockland, personal communication). It was published as part of a guide to management

of *G. pallida* in 1999 (Lane & Trudgill, 1999) and has been used widely ever since, as part of integrated management programmes, both in the short term in relation to chemical use, and the long term, in relation to crop planning and rotations (Table 3).

*Table 3. Previous Interpretation of soil sampling results for planning management of G. pallida (Lane & Trudgill,1999).*

| Result | Advice |
|---|---|
| No viable cysts found | Safe to grow potatoes without chemical treatment, but continue to sample soil regularly. |
| 1-10 eggs/g (LOW) | Apply a granular nematicide at 5 eggs/g or more, if growing an intolerant variety on very light soils. If G. pallida is present consider using granular nematicides as soon as detected. Apply a granular nematicide if cropping closer than 1 in 8 years, to limit PCN increase. Cropping with a partially resistant variety will limit PCN increase. |
| 11-60 eggs/g (MODERATE) | Apply a granular nematicide with a partially resistant variety to reduce crop damage and limit PCN increase. Highest populations may also need pre-treating with a fumigant. Increase crop rotation to at least 1 in 8 years. |
| More than 60 eggs/g (HIGH) | Use a soil fumigant or trap crop to reduce PCN infestation. Resample to check success of control tactics. Apply a granular nematicide with a partially resistant variety to reduce crop damage and limit PCN increase. Increase crop rotation to at least 1 in 6. |

However, feedback from advisers in the field reveals that thresholds now being used are different both in levels and context from those originally suggested, but this might be to reflect the field advisers' own experience with different varieties and different soil types, or perhaps the efficacy of the chemical being used for protection. Considering the importance attached to using varieties with a high level of resistance to either *G. pallida* or *G. ros-*

*tochiensis*, an important criteria is the presence of the particular species of PCN in the egg counts upon which the thresholds (for *G. pallida*) have been based.

The problem with using detailed criteria of eggs/g for action thresholds is that they are achieved as a result of several processes involving significant error (sampling, both in the field and often in the laboratory, the type of extraction process used, and the experience of the personnel involved in species identification and the number of (viable) eggs/g). Such processes involve many variables which together reduce the likelihood of being able to predict risk. PCN development may also be affected by soil type, the variety grown and other management practices; there is currently insufficient evidence on these factors to offer specific advice, other than to enact a PCN management programme in all potato fields, whether PCN has been detected or not.

*6.5 Revised thresholds for action in PCN Management*

Over the last 40 years, the problem of PCN in the UK, in particular *G. pallida*, has become increasingly economically important. It has been realised for many years that *G. pallida* increases most quickly when at low levels, with some trials work showing that population increase is almost as great in nematicide-treated plots as in untreated areas (Trudgill et al. 2003). In addition, with many susceptible potato varieties being grown once every 5 or 6 years, rotations are commonly too short to prevent *G. pallida* from progressively increasing. It is possible that the use of a range of thresholds has resulted in less impetus for action when PCN numbers appear to be low, but in fact the seriousness of these low numbers of eggs/g or viable cysts has not been appreciated, and has only exacerbated the situation.

To illustrate the need to take action at low levels, there follows a theoretical example, which does not consider other factors which might encourage the population to expand:
If we have detected 4 egg/g, and if we use multiplication rates observed in the field (lowest observed by Trudgill et al. (2014) was 46-fold, maximum 100-fold), this would result in 184 eggs/g to 400 eggs/g at harvest. Field-observed decline rates quoted in this Reference Guide range from 10% to around 50% per year, so, starting at 184 eggs/g, after 5 years rotation the eggs/g would theoretically be 110 eggs/g or 5.75 eggs/g respectively for the next crop. Thus there is a risk of increasing PCN even at low levels of multiplication, and we know levels of multiplication are highest at low levels of infestation. If we use 400 eggs/g as our post-harvest count, after 5 years we would have 237 eggs/g, or 12 eggs/g with

decline rates of 10% - 50% respectively. Thus low levels can become high levels very quickly and it is important to monitor PCN levels both at planting and at harvest to assess the risk of the chosen management programme in a field.

Thus it is essential that growers adopt a cautious approach to the management of PCN. All those involved in the industry, including buyers, processors, agronomists, farmers and growers, and those letting or renting arable land, need to adopt a new approach, not only with the use of resistant varieties, noting the effects of tolerance or intolerance, but also redefining thresholds for action. The management of PCN is complex, depending on a range of environmental factors, and unfortunately there are no thresholds that have been scientifically proven to provide the best trigger for action, depending as they do on several variables, such as sampling. It is vital that PCN is kept at low levels in a sustainable way, and Table 9 provides an illustration of the level of thresholds that must be adopted.

*Table 4. Revised Thresholds for Action*

| **Threshold** | **Action** |
|---|---|
| PCN undetectable | Adopt a cautious, preventative approach, utilising rotation and hygiene to prevent PCN spreading on a farm. Sample soil immediately after harvest to confirm PCN is still undetectable. |
| PCN just detectable; upto 5 eggs/g soil | Determine species present to take appropriate action regarding the chosen potato variety. Extend crop rotation. |
| PCN more than 5 eggs/g soil | Use a variety with a higher resistance rating. Extend crop rotation further. Consider the use of biofumigants and trap crops to prevent PCN multiplication and nematicides to preserve yield. |
| PCN more than 60 eggs/g soil | Consider the use of soil fumigants to reduce PCN levels. |

## *Chapter 7. Management options*

This Reference Guide recommends 'best practice' measures that will offer a sustainable strategy for PCN management in the long term, concentrating on the integration of management tools into an IPM programme. Bearing in mind the discussions in the foregoing sections, the main recommendations are set out in this chapter:

1. Sample soil to detect the presence of PCN and, if confirmed, determine the PCN species present and population levels to capitalise on aspects of biology that can be used to reduce population levels (7.2).
2. Use a rotation of at least 8 years to reduce PCN levels (7.2.1).
3. Use certified seed potatoes (7.2.2.1).
4. Ensure hygienic practices wherever possible (7.2.2.2. and 7.2.2.3).
5. Control potato volunteers (7.2.3.).
6. Use varieties resistant to the most dominant species of PCN, but ensure you are also aware of tolerance traits, which whilst maintaining yields might also increase population levels (7.3).
7. Consider the use of trap cropping (7.4) and biofumigants (7.5) in your rotation as additional tools in heavy infestations.
8. Use a nematicide if population levels are more than just detectable (7.6).
9. To determine the effects of any combination of the above recommendations, assess population levels at planting i.e. Pi (initial population at planting) and post-planting i.e. Pf (population at harvest). This will entail assessing numbers of eggs per gram, or at least a

### *7.1. Introduction*

Given the potential reproductive rate for PCN of 100-fold, management tools must, in theory, be 98% effective to prevent population increase, but in reality a combination of tools are required to produce an effective management programme.

Any management strategy needs to consider the long-term benefits of keeping levels of PCN at a minimum to prevent an increasing level of damage. As the potential host range is small, appropriate crop rotation and resistant varieties are a key component of control strategies. However, high levels of PCN require a higher percentage reduction than low levels of infestation, requiring more inputs, hence it makes sense to keep PCN levels low. Population increase is under control when Pf, the population at harvest expressed as eggs per gram of soil, is no greater than Pi, the number of eggs per gram soil at planting. Growers who monitor their PCN populations will thus be able to track the progress of their particular management programme.

Sustainable methods of control of PCN should always be considered first before chemical treatments are undertaken. Recent European legislation (EU Council Directive 2007/33/EC) has laid the foundation for the sustainable management of PCN at source (i.e. seed production), but commercial management also needs to develop sustainable cropping regimes to contain the spread of PCN. There should be a cost-benefit analysis of using such methods. Both long and short term measures need to be decided.

The need for management of PCN does not just relate to potatoes; the production of certain plants for planting (e.g. bulbs) require confirmation of the absence of PCN in places of production.

In the recent past, attempts have been made to provide a model to relate all environmental factors affecting the population dynamics of *G. pallida* and so provide reliable advice concerning a range of scenarios encountered by growers (Elliot et al. 2004). The public face of that Model is now The PCN Calculator, which is available on the AHDB Potato Division website (http://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator). However, the unexpected variability of a range of factors, and paucity of field data, have continued the use of the Model as an educational tool emphasising key principles for consideration rather than as a decision tool for farmers and agronomists. Trudgill et al. (2014), using regressions that form the basis of the PCN Calculator, found that there were major differences between field sites and varieties. When untreated, the yield of cv. Maris Piper was hardly affected in a highly organic soil with Pi >200eggs per gram, whereas the yield of resistant cv. Sante (resistance rating 4 for *G. pallida*, 9 for *G. rostochiensis)*, was decreased from a potential of about 60T per ha to about 20T per ha in a light silt with a Pi of 20 eggs per gram soil.

Such results support monitoring of PCN levels at a field level, so as to assess local conditions, rather than rely too heavily on theoretical estimates.

The Calculator/Model requires greater scrutiny and updating on factors such as the characteristics of potato varieties now in use, the genetic background of the population, varying decline rates, available nematicides and soil types. Thus for management decisions, the following sections should be consulted. Even for those fields where PCN has not been detected, a sampling regime should be maintained, especially after harvest, as well as measures to delay the introduction of PCN into fields, such as maintaining a good length of rotation, using only certified seed and ensuring good field hygiene.

### *7.2. Using knowledge of the biology and life cycle of PCN*

It is clear that PCN needs to be identified to species level if growers are to create effective management strategies. The following sections exploit aspects of PCN biology. If mixtures of species exist on any one farm, then the most cautious approach should be taken to prevent increasing population levels of *G. pallida*.

#### *7.2.1. Extend length of rotation*

Extending the length of rotation is a key element in control of PCN, and will, it is believed, help to compensate for the loss of nematicides and nematistats. From many observations, the natural decline rate for *G. pallida* is concluded to be less than that for *G. rostochiensis*, but other variables, such as soil type, are also influential. Monitoring population levels on a yearly basis will help to provide data for the optimum length of rotation.

It is clear that once PCN has been detected the population can potentially remain viable in that soil for at least 40 years, according to ongoing research. However, the natural rate of decline, when spontaneous hatching does occur even in the absence of a potato crop, is greatest in the first 10 years, meaning that crop rotation is regarded as a key element in PCN management. However, it will not be effective with the current commercial frequencies of potato cropping, which appear to average about 5 years. Recent research investigating rates of decline in the field show such a length of rotation is not always appropriate.

Table 5 combines the data collected for Table 1 and estimates for the number of years to bring populations down to 5 eggs per gram of soil, often considered the lowest level at which economic damage would be inflicted on an intolerant variety. Rates of decline recently reported in the literature or in anecdotal evidence for each species vary from 10% - 50% for *G. pallida* and about 30% for *G. rostochiensis*, thus suggesting rotations of at least 6.5 years may be necessary. As described in the section on decline, it is more appropriate for the decline rates in individual fields to be monitored.

*Table 5. Natural decline rates per year reported for PCN in general, G. pallida and G. rostochiensis in the UK, and estimated number of years required to reduce infestation levels to an acceptable level.*

| | **PCN in general** | ***G. pallida*** | ***G. rostochiensis*** |
|---|---|---|---|
| | | | |
| Trudgill et al. 2014 | | 15% - 33.5% (mean 26%) | |
| Lane & Trudgill, 1999 | 12% | 20% | 30% |
| Whitehead & Turner, 1998 | 20% - 49% | | |
| Stone et al. 1973 | | 15% - 24% | |
| Cole and Howard, 1962a | | | 32% |
| Cole and Howard, 1962b | 60% | | |
| Cooper, 1953; Grainger, 1964,1951 | | | 18% - 33% |

| | **P (eggs per gram of soil)** | | |
|---|---|---|---|
| Decline rate (% per annum) | 50 | 200 | 800 |

| | P (eggs per gram of soil) | | |
|---|---|---|---|
| 50 | 3.3 | 5.3 | 7.3 |
| 30 | 6.5 | 10.3 | 14.2 |
| 20 | 10.3 | 16.5 | 22.7 |

If the minimal length of rotations are not heeded, then additional measures such as nematicides, biofumigation and trap cropping need to be considered to reduce population levels, but if the feasibility of these measures decreases, so the need to concentrate on the length of rotation will increase. Some growers have realised this and are extending their rotations to 8 years or more, a step which is much more favourable to the next potato crop (after Trudgill et al. 2014). If the potato crop being grown is susceptible to PCN, or resistant but tolerant to it, then such use might also necessitate an extended rotation. However, the controlling factors and environmental conditions on each farm are different, so it is hard to be too prescriptive. Population levels in each cropping field should be monitored to assess the effectiveness of the prevailing management regime.

*7.2.2. Minimising Spread*

Minimising spread of PCN will also have benefits for a range of soil-borne diseases, and is fundamental for sustainable management programmes.

*7.2.2.1. Use certified seed potatoes*

To keep land free from new infestation, it is essential that farmers use seed that has been certified as grown in accordance with the Seed Potato Classification Scheme (https://www.gov.uk/the-seed-potato-classification-scheme). The growing of seed potatoes in the UK is subject to the new PCN Control Directive designed to minimise the risk of the introduction of PCN with planting material and to minimise its spread into clean land. Essentially this is achieved by growing seed potatoes on land found to be free from PCN and minimising the soil residues spread with the tubers. Farm-saved seed is subject to similar growing restrictions in Scotland, but not the rest of the UK, which may pose a higher risk of introduction, or an increase in PCN population levels outside Scotland unless farmers take similar precautions.

*7.2.2.2. Hygiene*

The industry needs to consider the financial benefits of raising the profile of hygiene measures. Recent anecdotal observations on the spread of soil-borne disease organisms (such as brassica club root) suggest that hygiene measures would have a significant effect for growers, both in the short and long term; contamination with disease organisms often shows earlier than with PCN. There should be economic benefits for any cleaning and safe disposal of soil waste, both for minimising the risk of infestation, yield loss, and reducing the need for pesticide applications.

Efficient management and containment of an infestation may be compromised by the ease with which cysts can be dispersed. They are moved from field to field by wind, flood water, farm machinery, implements and footwear, as well as with plants (other than potato) for transplanting (Anon, 1989). Plants for transplanting grown in infested soil may spread PCN cysts by the soil which adheres to their roots. Thus the greater amount of soil adhering to seed tubers, the greater the risk of PCN successfully establishing and reproducing in that area, since it is already in close proximity to its preferred host plant (Turner & Evans, 1998). The infective juvenile nematodes will only move a maximum of about 1 metre in the soil while trying to locate a suitable host. Thus the movement of man-made carriers and weather should be carefully controlled where possible and strict hygiene observed; cleaning machinery both before and after use, restricting movement of soil outside the field boundary and constructing natural windbreaks (Turner & Subbotin, 2013). Despite anecdotal evidence that soil residues on machinery are a major cause of infestations in clean fields, there are no standard hygiene measures for using machinery on farm land, or for machinery to be washed down between farms.

Potato boxes are traditionally made of timber, and whether they are slatted or close boarded may not be easy to keep clean. Covering potato boxes during transport to prevent spread is a seemingly simple measure that would also reap benefits.

Under the new PCN control Directive, potato processors need to have officially approved waste disposal procedures for soil residues before they can be used on agricultural land as a fertiliser or filling material. All waste, soil and by-products from potato harvesting, grading and processing operations should be disposed of in line with the Plant Health Code of Practice on Management of Agricultural and Horticultural Waste (http://webarchive.nationalarchives.gov.uk/20141216180052/http:/www.fera.defra.gov.uk/plants/publications/Guides/copManagementWaste.pdf). In Germany, the German Biowaste Ordi-

nance prescribes sanitation of organic waste before it can be used on arable land; cysts of *G. rostochiensis* have been shown to be killed by composting for 7 days at 50-55°C and by pasteurisation for 30 minutes at 70°C (Steinmoller et al. 2012). Boen et al. (2006) used 150L pilot scale reactors to determine that 8 days of composting where all the material had reached a temperature of 50ºC during the period, was the optimum for destruction of PCN cysts.

Washing or brushing tubers harvested from infested fields has always been considered a not very efficient method for those wishing to produce clean potatoes for sale, as many cysts lie in the cavities of new shoots or roots ('eyes'), but in some situations it may be effective; complete disinfestation was achieved on varieties Lizeta and Marfona from loam soils (Karanastasi & Kompi, 2011). Gardner et al. (2006) showed that all PCN cysts were removed from tubers of cv. Trent harvested from land infested with PCN when they were washed until less than 5% of tubers retained small visible soil patches, and were subsequently deemed to present an acceptably low risk of carrying PCN cysts to processing. However they suggested a wider range of soil types be tested to reinforce this finding. Certainly, even if soil residues remain on tubers, there is a low risk in heavily infested fields of spreading PCN to new locations as cysts may be found embedded around the 'eyes' (Dunnett, 1957). This risk may increase if the level of hatching increases with warmer weather (Vivian Blok, personal communication).

*7.2.2.3. Disposal of soil residues at harvest*

It has long been a standard recommendation to safely dispose of excess soil at harvest from crops in infested fields, but it is not routinely mentioned. Recent work in Belgium and elsewhere has suggested that such soil, if collected, can be used to detect PCN and collect cysts for speciation, and, with the available technology, different samples of excess soil can be traced back to different parts of fields to provide more detailed feedback (Goeminne et al. 2015).

*7.2.3. Control volunteer potatoes*

The adoption of length of rotation as a management tool can be affected by the persistence of volunteer potatoes, which are often difficult to control in subsequent crops. The use of the sprout suppression Fazor (maleic hydrazide) has been successful (http://uk.-dowagro.com/products/fazor/), as well as good weed control. At harvest, similar guidelines

are recommended as those applied for reducing potato blight i.e. harvest carefully, especially in wet conditions and make sure no rods on the harvester are missing.

In 1949, the English researcher Fenwick included woody nightshade, *Solanum dulcamara,* in a group of plants said to cause more than 80% hatch of PCN juveniles under suitable environment conditions (Fenwick, 1949). However, little evidence was found during research for this Reference Guide of an assessment of the ability of UK 'weed' species in Solanaceae, such as the nightshade family, to support populations of PCN, or to provide potential material for 'trap crops'. Recently commissioned research may produce new data.

*7.3.Use potato varieties resistant to PCN*

Use of varieties with high levels of resistance is considered the most highly effective control measure for suppressing PCN multiplication. They have the potential to increase national production, but as Table 6 shows, they do not appear at all in the top nine varieties currently being grown. Whilst it is appreciated that farmers or processors choose a particular variety for a number of commercial reasons, nevertheless the susceptible varieties most commonly grown can only exacerbate the *G. pallida* problem and in the long term prove to be unsustainable.

*Table 6. The resistance status of the top ten ware potato varieties grown in Great Britain in 2015 (after Swales, 2015).*

| Potato Variety | GB planted area (ha) | Resistance status against *G. pallida* Pa2/3,1 (rating) | Resistance status against *G. rostochiensis* (Ro1)(rating) |
|---|---|---|---|
| Maris Piper | 17,137 | Susceptible (2) | Resistant (9) |
| Markies | 6,426 | Susceptible (2) | Resistant (9) |
| Maris Peer | 4,805 | Susceptible (2) | Susceptible (2) |
| Lady Rosetta | 3,854 | Susceptible (2) | Resistant (9) |
| Melody | 3,623 | Susceptible (2) | Resistant (9) |
| Estima | 3,262 | Susceptible (2) | Susceptible (2) |
| Hermes | 2,895 | Susceptible (2) | Susceptible (2) |
| Pentland Dell | 2,716 | Susceptible (2) | Susceptible (2) |

| Potato Variety | GB planted area (ha) | Resistance status against *G. pallida* Pa2/3,1 (rating) | Resistance status against *G. rostochiensis* (Ro1)(rating) |
|---|---|---|---|
| Marfona | 2,379 | Susceptible (2) | Susceptible (2) |
| Harmony | 2,313 | Partially resistant (4) | Partially resistant (4) |

Chapter 4 explained the differences between the (GB) PVD and the European CPD, which both list details of the resistance of particular potato varieties to PCN. As explained, the procedures that lead to inclusion of varieties differ between the two databases; the GB National List lists varieties that have been independently assessed, whereas the European database relies on the information sent to it from a variety of sources. Field evaluations of the latter varieties have been done in the UK, but tend to lack replication in a range of soil types. Thus the advice given in either database should be considered as guidance only; other factors such as the PCN species characteristics and population level in the field, soil, aspect, and management practices will all affect the final yield.

Nevertheless, it is the aim of this Reference Guide to promote new varieties being developed and which show promise, both from a PCN-resistant point of view, and for particular market sectors. In most variety databases there is reference to particular pathotypes; tests for the (GB) PVD are done using standard reference populations. However, as it is the characteristics of individual field populations that are important and growers should be guided by the overall ratings for each PCN species, as given in Tables 6 and 7.

*Table 7. New varieties that are highly rated for resistance to G. pallida, with associated rating for G. rostochiensis, if known (data kindly provided by Agrico and HZPC).*

| Potato Variety | Use | Resistance rating for *G. pallida* | Resistance rating for *G. rostochiensis,* if known |
|---|---|---|---|
| Ambassador | Chipping, Crisping and Processing | 6 | 4 |
| Bartina | Traditional | 2 - 5 | - |
| Heraclea | Crisps | 6 | - |
| Innovator | Fresh; French Fries | 8 - 9 | - |
| Joly | Traditional | 5 | - |

| Potato Variety | Use | Resistance rating for *G. pallida* | Resistance rating for *G. rostochiensis,* if known |
|---|---|---|---|
| Leonardo | French Fries | 3 - 7 | - |
| Memphis | Traditional | 1 - 6 | - |
| Panther | Traditional; Fresh | 8 | 2 |
| Sifra | Traditional; Fresh | 1 - 6 | - |

It has been noted previously in this Reference Guide and in research work (Trudgill et al. 2014) that there are many environmental variables that might affect the perceived resistance rating for a particular variety. Recently Eric Anderson (Scottish Agronomy) and Jon Pickup (SASA) sought to provide more data on field evaluation of currently available varieties using replicated plots (Anderson & Pickup, 2015). They chose *G. pallida*-infested land at Luffness Mains, East Lothian, and planted varieties with varying levels of resistance alongside susceptible Maris Piper. The results showed that varieties with high ratings of 7-9 robustly reduced population levels with or without a nematicide, and that the data strongly supports resistance scores obtained using pot tests as done for the GB National List.

A caveat on the use of resistant varieties in general is that it is also important to have knowledge of whether a resistant variety is tolerant or intolerant of PCN attack. If tolerant then the level of PCN may well increase, even though yield may be protected, whereas if it is intolerant then yield may well be affected if PCN levels are high. Unfortunately, as described earlier, there is little data concerning the tolerance rating of varieties, so a cautious approach is advised. Applying chemical control at planting may reduce the potential increase in PCN levels from growing resistant but tolerant varieties.

If susceptible varieties are grown then advice on other aspects of control needs to be adjusted. For example, a susceptible variety would allow the PCN population to increase several-fold, thus increasing the subsequent risk of damage and requiring an extended length of rotation to compensate.

### *7.4.Trap cropping*

Occasionally potatoes have been planted as a trap crop, with the aim of harvesting them before cysts mature. In Belgium, early harvesting is part of a preventative management approach for very early potato varieties but recent observations indicate that both *G. pallida* and *G. rostochiensis* are developing earlier than would be assumed by conventional data, emphasising the need for local monitoring of the effect of temperature on life cycle before adopting this method (Ebrahimi et al. 2014). Turner et al. (2006) investigated the trap crop concept in a range of field trials from 1999 to 2002 that evaluated 10 clones of either wild *Solanum* potato species, breeders' hybrid lines or commercial varieties. All had high resistance to all known PCN pathotypes (both *G. rostochiensis* and *G. pallida*) and the ability to stimulate high levels of PCN hatch. The work showed the potential for the development of some clones as a means of reducing high PCN field populations, especially in organic situations. However, recent research into the life cycles of PCN in the field suggests this method may be more hazardous than first thought because of 'climate change' affecting the time of formation of cysts (Ebrahimi et al. 2014). However, it is suggested some reduction in the PCN could occur if the crop was harvested at the right time, using sound knowledge of life stage development data for particular fields.

A better approach would be to use a crop that stimulated PCN hatch, but which did not allow PCN to multiply. There have been several investigations into potential candidates, but the plant attracting most attention in the UK and elsewhere over recent years has been *Solanum sisymbriifolium* (sticky nightshade, SN), a non-tuber bearing solanaceous plant which appears to have a high production level of hatching agents, but which significantly reduces the potential of PCN to reproduce.

Studies of this plant have been done in the UK and have recently commenced in the USA (Dandurand, et al. 2014a). PCN juveniles were shown to invade SN but did not produce mature females or cysts. This supported observations by Sasaki-Crawley et al. (2010), who found that whilst the hatching factor of *S. sisymbriifolium* differed from that produced by *S. tuberosum*, with solanoeclepin A not detected, it tended to be more attractive to J2s. However, the promise shown by this plant needs to be tempered by its apparent limitations; Timmermans et al. (2009, 2006, 2005) showed that SN had strong temperature limitations to its growth, not emerging at temperatures below 8ºC, and needed a certain cropping time to produce sufficient growth to exert its effects on PCN; the crop needs to accumulate at least 700g/m$^2$ of dry matter to achieve sufficient nematode control. In that context, a model using field data from 2002 showed that the crop could reach the required

minimum size in the UK, but maybe not in the north of the UK. In The Netherlands, the above-ground growth of *S. sisymbriifolium* was deemed to be adequate if planted between early May and the end of July (Timmermans et al. 2007; Timmermans, 2005). Szymczak-Nowak et al. (2007) assessed SN in a field in Poland; the crop was sown in May and cultivated until the end of September, and was found to reduce PCN by 61-77.9%; Timmermans (2005) recorded an average reduction of PCN of 75%; Malinowska et al. (2005) achieved an average reduction of 72% in the PCN juvenile population, most at a density of 25-30 plants/$m^2$. Climate change might alter and extend barriers, but it might also be regarded as a 'step' change, as in 2015 temperatures in some parts of the UK have been cooler than usual.

*S. sisymbriifolium* seed is now marketed by Greenvale as DeCyst, and Branston as Foil-Sis. Greenvale advise DeCyst is the only scientifically proven trap crop control method for PCN, and that it has been used commercially in the UK for the last 10 years, being very effective at reducing PCN, and contributes in excess of 12 tonne/ha of green manure. The company also states it has worked with the James Hutton Institute to breed and select new varieties that germinate in cooler conditions (8-10ºC) e.g. variety Trickster. However, it is not clear how this variety differs from varieties used earlier. Further instructions and details are given on the Greenvale website (Anon, 2015a). Disadvantages listed in Defra Report HH3111TPO_1748_FRP (Kerry et al. 2003) that need to be reviewed are: the crop is slow to establish, prefers low pH, has reduced growth in sandy loam soils, may require irrigation to establish, requires fertiliser application and weed control, requires agronomic management, and efficacy is variable depending on the PCN population and the thermal activity threshold. Despite anecdotal evidence that germination of DeCyst is problematic in northern UK, and difficult to fit into rotations, there is a consensus view that, in some areas, *S. sisymbriifolium* may prove to be a very useful tool for reducing PCN population levels.

These plants also appear to have a detrimental effect on other nematodes, such as *Pratylenchus goodeyi* (Pestana et al. 2014) and *Meloidogyne chitwoodi,* but not *M. arenaria, M. hapla* or, in some cases, *M. javanica* (Dias et al. 2012). Thus the effect of SN on a range of species known to attack potato needs to be investigated further.

Defra (England and Wales) and AHDB Potatoes are jointly funding a 3-year project (2013-2016) for ADAS to investigate the potential of both native and non-native *Solanum* species as Potato Cyst Nematode trap crops (PS2149/R468), identify the most likely species as

potential trap crops for PCN under UK conditions, and quantify the agronomic requirements for growing and destroying the trap crop. Clearly the economics and suitability of trap cropping in rotations need to be reviewed with stakeholders, but the potential for such methods appears very favourable as part of a sustainable, integrated control programme.

### 7.5. *Biofumigation*

#### *7.5.1. Introduction*

'Biofumigation' is a term that was first coined by Australian researcher John Kirkegaard to describe the suppression of soil borne pests, pathogens and weeds by biocidal compounds (principally isothiocynates) released in soils when glucosinolates in brassica crop residues are hydrolysed. Practically, biofumigation is achieved by growing brassica cover crops such as Indian/brown mustard (*Brassica juncea*) and oilseed radish (*Raphanus sativus*), typically for a period of 10-14 weeks if grown during the summer months. Several other plants have the potential to act as biofumigants against PCN, but research has shown they can also act as stimulants for hatching, which might have benefits but might not. Some plants produce effects that fall into the category of allelopathy**,** a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. Alfalfa (*Medicago sativa*) is an example of this. It has been used successfully to control *G. rostochiensis* in in-vitro studies, but it is susceptible to frost; it contains saponins, mainly consisting of triterpene glycosides of mediagenic acid, which possesses several biological properties including a biocidal activity on different soil micro-organisms (D'Addabbo et al. 2012).

Certainly, the use of biofumigants has soared since the 1990s, maybe largely due to the marketing of a plethora of commercial products. Many pot experiments have been done, but a review of research results shows that results in field situations are often very different, illustrating our relatively poor knowledge of the soil environment. An independent analysis of their effectiveness and cost-benefit needs to be made. The following section reviews the biofumigation process, research on biofumigation for PCN reduction and practical application on a field scale.

#### *7.5.2.The biofumigation process*

*Table 8. Examples of brassica species that are recognised to produce high quantities of GSLs in their tissues*

| Species | Common name | Examples of commercially available varieties | Applicable for PCN management |
|---|---|---|---|
| ***Brassica juncea*** | Indian mustard<br>Caliente mustard<br>Brown/yellow mustard | Caliente 99 or 199<br>Scala<br>Vitasso<br>Spudguard | Yes |
| ***Raphanus sativus*** | Oil/oilseed radish | Bento<br>Doublet | Yes |
| ***Eruca sativa*** | Rocket<br>Arugula | Nemat<br>Trio | Limited data |
| ***Brassica carinata*** | Ethiopian mustard<br>Abyssnian mustard | Carbon<br>Cappuccino | Limited data |
| ***Sinapis alba*** | White mustard | Smash<br>Architect<br>Vitaro | Limited data, often used in combination with other species |
| ***Brassica rapa*** | Canola<br>Turnip rape | | Limited data |
| ***Barbarea verna*** | Land cress | | No data |
| ***Nasturtium officinale*** | Water cress | | Limited data |

A number of plant species belonging to the brassica family (*Brassicaceae*) are known to produce high concentrations of glucosinolates (GSLs) (secondary metabolites) in their roots and foliage (Table 8 and Figure 12). Glucosinolates, whilst non-biocidal, are the integral pre-cursor required for the production of volatile compounds that suppress pests and pathogens. To date, around 120 GSLs have been identified and brassica species and varieties vary in their GSL profile and content. Additionally, glucosinolates differ both quantitatively and qualitatively between different plant tissues, particularly when foliage and roots are compared. There are three major types of glucosinolate; aliphatic, aromatic and indolyl (indole). Indolyl glucosinolates do not produce isothiocynates.

Biofumigation is achieved by liberating the GSLs from the cell vacuoles through tissue damage i.e. flail mowing. From separate cells (myrosin cells), an enzyme known as myrosinase (thioglucoside glucohydrolase) (MYR) is released. In the presence of water MYR catalyses the conversion of GSLs into a range of volatile compounds which include isoth-

iocynates (ITCs), thiocynates, epithionitriles, nitriles and oxazolidine-2-thione. Whilst each of these compounds are known to have biocidal properties, the majority of research has focused on the isothiocynates which have lower volatility.

**Figure 12** *Common species of biofumigants investigated for PCN management; A =* Sinapis alba *(white mustard), B=* Eruca sativa *(Rocket), C =* Brassica juncea *(Indian mustard) and D=* Raphanus sativus *(Oil radish)*


*7.5.3.Factors affecting biofumigation*

The relative performance of biofumigation can be variable, and depends on a range of environmental and agronomic factors. A preliminary consideration that needs to be taken into account is the species and variety of biofumigant used. It is important to select a species/ variety that produces high quantities of glucosinolates known to be effective against the target pest or pathogen. As a guide, preference should be given to species that produce GSLs such as sinigrin (2-propenyl glucosinolate), gluconasturtiin (2-phenylethyl glucosinolate) and glucotropaeolin (benzyl glucosinolate). For instance, certain varieties of Indian

mustard (*B. juncea*), E.g. Caliente 99, contain high quantities of sinigrin in their foliage. Hydrolysis of sinigrin results in the production of allyl-isothiocynate which is reputed to be the most biocidal isothiocynate by numerous sources.

According to the findings of Ngala et al. (2014) it is preferable to sow biofumigants in the summer months (June-August) following the harvest of cash crops such as combining peas, oilseed rape, winter barley and winter wheat. There are a number of reasons to support this practice. Firstly, biofumigants such as Indian mustard are highly susceptible to sub-zero temperatures associated with ground frosts. Additionally, biofumigants produce more biomass when the photoperiod is longer and the temperatures are intermediate (15-25$^{o}$C). Although there are some exceptions, most studies concur that brassica species grown in longer days with intermediate temperatures, high light intensity, and dry conditions have the highest total GSL content (Bjorkman et al. 2011).

The seed rate and subsequent plant density of biofumigants may be important in the biofumigation process. Whilst this aspect has received little attention, an AHDB funded HAPI (Horticulture and Potatoes Initiative) project has been investigating GSL accumulation in a range of biofumigants, grown at contrasting seed rates, under field conditions. Until this data is available, it is advisable to follow the guidelines provided by the seed supplier.
Total glucosinolate concentration increases with increasing sulphur supply as do specific glucosinolates such as sinigrin and glucoraphanin (a major glucosinolate found in oil radish). Most seed suppliers recommend sulphur inputs between 25-60 kg/ha. Increasing N inputs can, however, reduce aliphatic and aromatic glucosinolates when low sulphur inputs i.e. 10-20 kg/ha are supplied. Modest inputs of nitrogen (c.100 kg/ha) are likely to have positive effects on biofumigant biomass.

Biomass incorporation is a critical component of biofumigation. There is universal agreement that the above-ground biomass needs thorough pulverisation to enable the greatest release of glucosinolates and myrosinase. Incorporation should take place at mid-flowering as many sources report higher GSL concentrations in the foliage at this time, but the HAPI project may tell us more. The main implement used in biomass maceration is the flail mower (Fig. 13). Following maceration, the brassica residues need to be incorporated in quick succession, as the greatest proportion of volatiles (including ITC's) are released within the first 2 hours after maceration. Incorporation is best achieved using a rotovator or spader which will enable thorough mixing of the soil. Following incorporation, it is advis-

able to rapidly compress the soil using heavy rolls. This process, should help create a seal and therefore retain ITC's for longer. Incorporation is best undertaken when the soil is moist to improve soil sealing and potentially increase glucosinolate hydrolysis. Further work is currently being undertaken to understand the factors affecting incorporation.

**Fig. 13.** *Incorporation of biofumigants showing A = maceration using a flail mower, B= incorporation with a rotovator, C = incorporation and soil sealing with a spader and D= soil sealing with Cambridge rolls*


*7.5.4. Summary of work investigating biofumigation for PCN management*

Whilst further work is still required to fully understand the factors effecting biofumigation, the following recommendations can be made: -

1. The best time to grow biofumigants is June-early November. Overwintering, has shown to be less successful and some species E.g Indian/brown mustard are susceptible to frost
2. Indian/brown mustard and oilseed radish have consistently shown to have a suppressive effect on PCN. Other species such as rocket have been shown to have potential.
3. Biofumigants require nitrogen for biomass and Sulphur to aid production of GSLs
4. Thorough pulverisation of biofumigant biomass is required prior to incorporation. Chopping, incorporation and pressing need to be undertaken in quick succession to avoid loss of important active (volatile) substances

Whilst the application of biofumigation for potato cyst nematode management has been studied since 1925, it has received far greater attention in the last 15 years (see Table 8), perhaps due to diminishing nematicide options and pressure from tightening EU pesticide legislation (Regulation (EC) No 1107/2009). From the literature, there is a general consensus that ITC's, particularly 2-propenyl and 2-phenylethyl, are nematicidal and also have an inhibitory effect on hatching under *in-vitro* conditions. Performance under field conditions, however, is not consistent between publications. This may be explained by the selection of species/varieties and differences in the methodology. Ngala et al. (2014) recorded a consistent reduction (47 - 95%) in field populations of *Globodera pallida* when Indian mustard and oilseed radish were grown during the late summer and incorporated in the autumn. Results were less consistent when biofumigant species were overwintered. Research elsewhere in Europe suggests that it is difficult to reach sufficiently high levels of toxicity to reduce hatching of *G. pallida* using *Brassica juncea* genotypes under realistic conditions. However, research results are variable. Pastuszewska et al. (2013; 2010) showed in field experiments that 'white mustard' (*Sinapis alba*) varieties could reduce *G. rostochiensis* infestations by 30%-50% (depending on variety) when sown as a stubble catch crop. However, Nowakowski & Franke (2013) showed varieties of 'white mustard' differed significantly in their yields and nematicidal activity, but all limited the development of PCN infestations to some extent. The smallest effect occurred in the year of highest nematode density and smallest root yield of white mustard. However, Brolsma et al. (2014) found poor control of *G. pallida* by the maceration and incorporation of Brassicaceae into soil, and suggested this was most likely due to lower concentrations of 2-propenyl isothiocyanate under field conditions compared to the laboratory. Valdes et al. (2011; 2012a) using in vitro assays and pot tests containing *G. rostochiensis*, found no benefit from root diffusates and plant extracts of *Sinapsis alba*, *Brassica napus* and *Raphanus sativus,* and later field results confirmed this. There was evidence of enhanced root hatch of *G. rostochiensis*, also reported elsewhere. Valdes et al. (2012b) also investigated whether root diffusates and extracts of green manures from Brassicaceae, in particular 'yellow mustard', fodder radish and rapeseed, have a direct effect on eggs and J2s, and found these did not significantly

affect egg shell permeability, although some internal effects on the nematodes were observed.

Thus the work on the effectiveness of biofumigation continues, so as to provide more conclusive data in a range of situations; a summary of key publications on biofumigation to date are set out in Table 9.

**Table 9.** *A summary table showing the key publications on biofumigation in relation to potato cyst nematode management*

| Publication | PCN spp. | Key Findings |
|---|---|---|
| Morgan (1925) | - | Preliminary observation of PCN suppression from mustard |
| Triffit (1929) | - | White mustard found to cause reduced populations of PCN in small non-replicated pot-based experiments |
| Smedley (1939) | - | Demonstrated a suppressive effect of 'phenyl-isothiocynate' on PCN under field conditions |
| Ellenby (1944) | - | Hatching of PCN inhibited by root diffusates from black mustard (*Brassica nigra*), white mustard cress and solutions containing allyl-isothiocynate. This effect was found to be non-reversible for white and black mustard. |
| Ellenby (1945) | - | Field experiment demonstrated a 100% increase in potato yield when black mustard oil is applied to PCN infested soil |
| Ellenby (1951) | - | Further field experiments supporting the 1945 publication by the same author |
| Pinto et al. (1998) | *G.r* | *In-vitro* study showing 100% mortality of *G. rostochiensis* juveniles when exposed to 2-propenyl glucosinolate at 1 mg/ml with myrosinase (25%). Exposure to the GSL without myrosinase had no effect on nematode mortality. |
| Buskov et al. (2002) | *G.r* | *In-vitro* study investigating the effect of 8 GSLs with and without myrosinase on *G. rostochiensis* juvenile mortality. Intact GSLs had no effect on mortality. However, 2-phenylethyl glucosinolate at 1mg/ml with myrosinase caused 100% mortality within 16 hours. A similar effect was observed for benzyl and 2-propenyl following 24 and 40 hours of exposure respectively. |
| Serra et al. (2002) | *G.r* | 2-phenylethyl glucosinolate at 1 mg/ml with myrosinase caused 100% mortality of juveniles within 8 hours. |
| Aires et al. (2009) | *G.r* | Growth chamber experiment evaluating effect of brassica extracts (kale, broccoli, cauliflower, turnip, cabbage and watercress) applied to a PCN infested potting medium. All brassica extracts caused a significant reduction in newly formed cysts on potatoes cv. Desirée when compared to the control. |
| Lord et al. (2011) | *G.p* | Leaf extracts from 12 brassica accessions caused a significant increase in mortality. Oilseed radish, Indian mustard, watercress and white mustard caused the highest reductions.<br>The mortality of *G. pallida* encysted eggs were found to increase by >95% in a pot-based experiment where *Brassica juncea* cv. Caliente 99 was chopped, incorporated and sealed into PCN infested soil. |

| | | |
|---|---|---|
| Valdes et al. (2011) | *G.r* | Pre-exposure to root diffusates from *Brassica napus, R. sativus* and *S. alba* found to have a stimulatory effect on hatching. No nematicidal effects seen. |
| Valdes et al. (2012) | *G.r* | Biofumigation with a white/yellow mustard (*S. alba*) cv. Zlata did not cause a reduction in the field population, infectivity or hatching of *G. rostochiensis* |
| Ngala et al. (2014) | *G.p* | Two years of field based experiments where PCN populations were monitored in plots where oilseed radish cv. Bento, Indian mustard cv. Caliente 99 and rocket cv. Nemat were either grown July-October or September-March. The viability of encysted PCN eggs was significantly reduced in plots with Caliente 99 and Bento pre-incorporation (partial biofumigation) and post incorporation in the July sown experiments (2011 and 2012). However, no significant differences were observed in the September sown experiment. |
| Watts et al. (2014) | Mix | Suppression of PCN observed in a field experiment |
| Ngala et al. (2015a) | *G.p* | Cysts of *G. pallida* pre-exposed to root and leaf extracts from *B. juncea* cv. Caliente 99 and *R. sativus* cv. Bento and then transferred to potato root diffusate to assess hatching. Pre-exposure to root and leaf extracts of Caliente 99 at ≥0.3 mg/ml completely inhibited hatching. Leaf extracts of Bento did not completely inhibit hatching and inhibition was only seen at higher doses (≥0.5 mg/ml). However, pre–exposure to root extracts of Bento resulted in complete inhibition of hatching when the concentration was ≥0.25 mg/ml. |
| Ngala et al. (2015b) | *G.p* | Presents experiment which support ‘partial biofumigation’ observed in their 2014 publication. |

## *7.6.Chemical control*

The collected evidence suggests that the use of nematistats or nematicides do not routinely result in improved yield, or a significant decrease in the level of PCN infestation, unless PCN levels are very high. They work best at low levels, and help to manage populations when tolerant varieties are used. Results, however, vary from site to site, and the situation is complex, with chemical control depending on many factors, including the characteristics of the product itself, the hatching period of the PCN species, soil type, soil pH, drainage, the characteristics of the varieties grown, and efficiency of incorporation and use. Growers are advised to monitor the Pi and Pf of the populations in their own fields to assess the effectiveness of chemical control as a management tool. Certainly, if chemicals are used they should be applied as efficiently as possible, at planting, as soon as PCN is detected in a field.

There is an ever-decreasing range of chemical control products for nematodes, with losses in recent years including aldicarb (Temik10G) and methyl bromide. At the time of writing (2015-2016), oxamyl (Vydate) may be in short supply for 2016, but is anticipated to be available for 2017. However, they should never be considered a panacea because unfortunately, for many reasons, they only offer partial control.

Chemical control needs to target the most vulnerable stage of PCN, the infective juveniles, during their migration from the protective cyst to the potato root, and ideally prevent them from entering the root where they develop and multiply. During this phase the outer cuticle (skin) of the nematode is in direct contact with soil solutions and will actively absorb any chemicals within those solutions. Thus most chemical control is done by incorporating one of the three currently approved granular nematicides which are dissipated in this soil solution. These chemicals are by their nature toxic to the nematode but need also to be non-phytotoxic to the crop and, if systemic, need to leave no residues in plant material.

The current granular nematicides are the oximecarbamate Vydate 10G®[1] (10% oxamyl) from DuPont®, and the organophosphates Nemathorin®(10% fosthiazate) from Syngenta® and Mocap 15G (15% ethoprophos) from the Certis marketing company. This Reference Guide does not compare the efficacy of products used for PCN because of the variability in results observed. Furthermore, Mocap 15G has recently replaced Mocap 10G, and has significantly reduced the amount of active ingredient being applied from 10kg ethoprophos/ha to 6kg ethoprophos/ha, thus making comparisons difficult.

These nematicides are applied at planting, to target PCN juveniles as they travel through the soil to growing potato roots, and at the very least delay an attack. The actual period of

[1] There will be no supplies of Vydate 10G® in 2016, but sales should resume in 2017. Contact Dupont® for further details.

persistence and effectiveness of such products, however, is influenced by many factors, such as temperature, soil organic matter, soil pH, soil type and composition, soil water and possibly microbial transformation. Thus the recorded persistence (or 'half-life' as it is often quoted in scientific papers) of a product is only a guide as to its persistence in any particular field. The quoted half-life for Nemathorin, for example, ranges from between two and six weeks (Qin et al. 2004). The quoted half-life for oxamyl as Vydate, however, is extremely variable, dependent on the temperature at planting, and whether soils have previously been treated with this product. In particular, the persistence of oxamyl appears to increase with greater acidity, e.g. from 1.5 - two weeks at pH above 7.0, to sixteen weeks at pH 4.8 (Anon, 2005). The peak of egg hatch of *G. pallida* has been estimated as six to seven weeks after planting, with an extended hatch of up to 12 weeks, compared to a peak of about three to four weeks for *G. rostochiensis* over a period of about six weeks. Thus against *G. pallida*, some products may not be effective for the entire hatch period. In addition, if there were second generations as a result of an anticipated change in weather patterns (through 'climate change' or other effects), there would be no chemical remaining to exert control.

Pesticides control pests in a variety of ways, depending on their chemical structure and characteristics. It is important to understand how individual products work in order to use them most effectively, and to understand their limitations.

A nematistat is a term used to describe effects when nematodes are exposed to low concentrations of organophosphates (ethoprophos, fozthiazate) and carbamates (oxamyl). These products do not always kill nematodes, but appear to disorient, paralyse, or confuse nematodes and so prevent or delay infestation of roots. If the product is present for a long enough period of time, the nematodes will eventually starve to death, but it is also likely that some nematodes will regain their ability to find and penetrate roots.

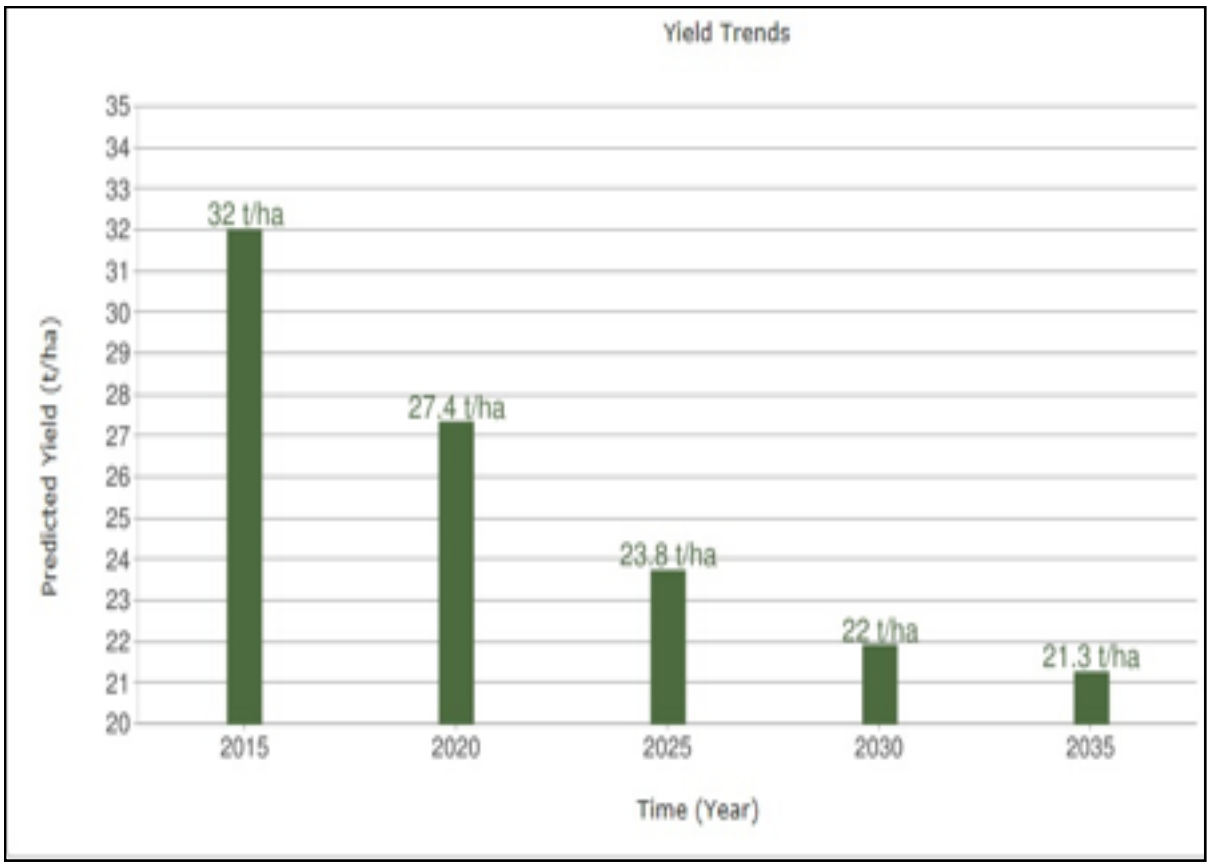
In reality, the same product could be acting as both a nematistat and a nematicide, the latter able to kill nematodes in the same situation. Keeping in mind that organophosphates and carbamates must be distributed through the soil via irrigation water or mechanical incorporation, any particular application will likely result in a range of concentrations depending on the uniformity of incorporation. In some areas, concentrations will likely be high enough to kill nematodes (nematicidal effect), while in others sublethal effects will occur (nematistat effect). Fosthiazate is stated to have an initial nematostatic effect, which stops

the nematode movement in the soil, and a consequent nematicidal action (Nieto et al., 1998).

The persistence of the chemical product can vary according to several environmental factors such as soil type, soil pH, temperature, and the amount of precipitation or irrigation, which may delay the effect of the pesticide if it is dry, or wash it away if there is significant rainfall. If low soil temperatures occur after planting, a delay in peak hatching of the nematodes and a delay in plant emergence may result in the main hatch occurring when little chemical remains at lethal concentrations. This would mean reduced protection to the plant and thus a reduction in the level of invasion. There is no evidence that the granular pesticides used at planting affect eggs inside the cyst, but fumigant pesticides are more likely to have some effect.

Some nematicides are classified as non-fumigants and may be formulated as granules, emulsifiable concentrates or liquids. In contrast to fumigants, these compounds have a low volatility, so they do not evaporate out of the soil rapidly and do not require any form of soil sealing. However, this also means that these compounds do not volatilize and therefore only control nematodes where they are placed or where they are moved to by water or tillage. They are highly toxic, so low dose rates are effective and there is often a considerable amount (90%) of inactive ingredient included to help facilitate dispersal of the product. Most compounds have very low phytotoxicity, so they can generally be used immediately before planting.
The PCN model on the AHDB Potatoes website can also be used to illustrate the trend of potential benefits of using nematicides (Fig. 14).

*Figure 14. Illustration of predicted yield of Estima, intolerant non-resistant, with an initial population of 20 eggs/g soil G. pallida, potential 40 t/ha yield, plus nematicide and a 5 year rotation (PCltd, 2015b).*




These predictions provide a theoretical illustration that compared with the use of no nematicide at all, the use of a nematicide could potentially increase yield by about 50% in the first year, more in subsequent years.

For less tolerant varieties, yield benefits are most likely to be seen when nematicides are applied at lower PCN population levels. For example, varieties such as Maris Peer, Marfona, Santé and Lady Rosetta showed yield increases when a nematicide was used and are therefore deemed intolerant (Kerr, 2007).

However, over the years there has been mixed results from the use of chemical products, although many trials are arguably not relevant in 2016, either because the products have changed or even been withdrawn, or because the economics of chemical use has itself changed. Brown (1983) used 13 trials across the Midlands with a wide range of soil types, infested with both *G. pallida* and *G. rostochiensis.* He concluded that whilst in general yield losses were largely recouped by the nematicide treatment applied, there were a significant number of trials which gave no correlation between potato yield and nematode density, and an unpredictable response to nematicide. It was postulated that the variable performance could be linked to other factors such as an interaction with the fungal organisms

*Rhizoctonia* or *Verticillium*, or the presence of other plant-parasitic nematodes, adding another potential confounding factor to the response to nematicide application.

Minnis et al. (2004) demonstrated significant ware yield increases from 22 to 35t/ha with granular nematicide applications in one experiment with initial population densities of 70-131 eggs/g soil; a second experiment at a mean of 190 eggs/g soil showed only marginal benefits. Where a soil fumigant was added into these experiments significant yield improvements were achieved, especially with the variety Estima. Trudgill et al. (2014) conducted an intensive range of trials to aid validation of the PCN Model (used in the online PCN Calculator) and came to the conclusions that nematicides did not increase yield potential, but it was noted that this was very site dependent. On a peat soil with 200 eggs/g soil the yield of cv. Maris Piper was hardly affected whereas the yield of cv. Santé was reduced from a potential of about 60 t/ha to only 20 t/ha on a light silt with an initial population of 20 eggs/g soil. Thus it seems the benefits of using nematicides are variable, but this may not be surprising given the influence of several environmental factors and different management practices. Organophosphates are, in the main, lipophilic and are adsorbed onto organic matter so high OM soils will reduce their effectiveness (Bromilow, 1980). As stated earlier, nematicides/nematistats may have a role in keeping PCN levels down on resistant, tolerant (and intolerant) varieties.

Of the products currently available, oxamyl has both contact and systemic action and the systemic nature of this chemical has both positive and negative effects. Positive because it can work inside the plant and thus lead to reduced feeding and moulting but negative because there is a higher risk of chemical residues in the plant residues, consequently the harvest interval (HI) is important. Fosthiazate and ethoprophos are organophosphates (contact only) are rapidly broken down in plants and generally leave no detectable residue. Advice on the harvest interval for products, as well as sampling protocols for tuber residue testing, is available in product stewardship guides produced by the chemical companies.

Carbamate nematicides such as oxamyl inhibit the enzyme acetylcholinesterase in nerve tissues which then impairs neuromuscular activity disrupting nematode movement (Evans & Wright, 1982). At field concentrations their effect is mainly to disrupt the orientation behaviour of nematodes, or paralyse them. The product can have a reversible effect, whereby the infectivity of J2s is maintained after nematicide activity has declined. Wright et al. (1989) found that the reserves of neutral lipids (nematode ‘fats’) of infective juveniles of *G.*

*rostochiensis,* incubated in field rates of oxamyl for between 8 and 35 days, were significantly greater than controls maintained in water and, on recovery after transfer to water, their infectivity was not impaired. *G. pallida* infective juveniles appear to utilise their fat reserves at a significantly lower rate than *G. rostochiensis*, thus giving them an advantage when searching for feeding sites (Robinson et al. 1987a; 1987b) and the prospect of a higher success rate. This, combined with an extended hatching period for *G. pallida*, helps to explain why such compounds sometimes appear to be ineffective.

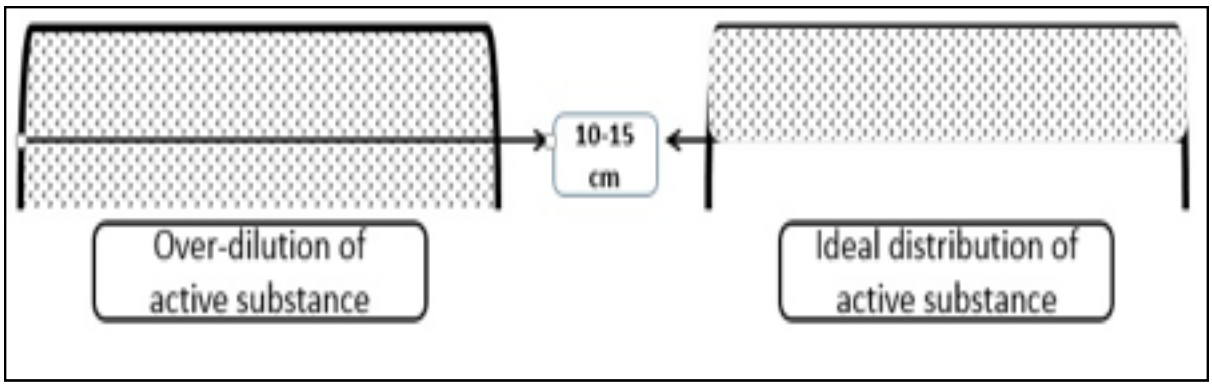
Other factors such as the distribution of the product, soil pH, temperature or moisture can all affect performance and rates of chemical used. Soil pH influences the persistence of oxamyl with guidelines from DuPont (2015) for Vydate® 10G suggesting that for soil pH < 6.0 growers are recommended to use residue tests at harvest and for those soils and soils at pH > 6.0 a minimum of 80 days must elapse from the Vydate® 10G application to the point of initial haulm destruction and/or lifting, whichever comes first. At pH 8 and above the activity of oxamyl may also be negatively affected but as it is hydrophilic it is not affected in high OM soils (Whitehead, 1988). Soil temperatures also affect persistence with cold soils reducing soil activity, giving greater persistence but more residue problems, and warmer soils increasing the activity leading to reduced persistence. Adequate soil moisture is required to put the chemical into soil solution around the nematode at the required lethal dosage. Too little moisture, resulting in dry seed beds, will prevent its dispersion, whereas too much soil moisture can lead to over-dilution and leaching into groundwater especially for the more water soluble products. The heavier clay soils have very good water-retention properties but can coalesce to form dense structures when dry, thus making seed beds cloddy. The distribution of chemical product in such soils is very poor, leading to ineffective nematode control.

The rate of products and indeed their efficacy in different soil types, should also be considered (e.g. Anon, 2015b). Many organophosphates such as ethoprophos are lipophilic and as such require intimate mixing with the soil (i.e. rotary cultivation) and are of little use in very organic soils, such as peaty loams, due to adsorption (Whitehead & Turner, 1998). Oximecarbamates, such as oxamyl, are hydrophilic and work effectively in a range of soils, including organic soils. They are less effective or ineffective in very alkaline soils (pH>8.0) and also degrade faster at high soil temperatures.

So it is critically important that granular nematistats/nematicides are applied correctly as poor incorporation will reduce their benefits. It is also essential for them to persist at suitable concentrations for long enough in the soil but not so long that residues are problematic. Attention to correct incorporation will produce benefits. The form of the nematicide is small dust-free micro granule, approximately 1mm in diameter. The rate of application depends on the product but using full rate Vydate® 10G as an example, 55 kg/ha, will need to be incorporated to a maximum depth of 15cm. This means 55 kg is therefore diluted in to1500$m^3$ (approximately 1800 - 2250 tonnes) soil or just 5g over 1$m^2$ (150 litres soil) when incorporated to 15cm depth. Knowing this it can be envisaged that correct depth and distribution in the soil is paramount to achieving good control. As the granules need to be incorporated thoroughly to get lethal soil concentrations of the chemicals they need good horizontal and vertical distribution in addition to being applied at or close to planting of the crop. Research carried out by Woods (1997) demonstrated how the use of bed-tillers, web and star stone separators and planting equipment can influence the incorporation with some causing dilution or banding effects. These effects did not appear to greatly reduce PCN control or crop yield but could also depend in part on the potato variety.

DuPont (2015) recommend the use of rotavators which work in the vertical plane, rotary diggers, rather than the horizontal plane, reciprocating or rotary harrow type. Rotary diggers have an aggressive mixing action which involves the soil being physically lifted and mixed whereas the reciprocating or rotary harrows tend to move or stir the soil. As nematicides are also incorporated on stone/clod separators DuPont (2015) also suggest that extra care should be taken when using the star separator, rather than the web separator, as banding of the granules may occur thus reducing the control achieved.

*Fig. 15. Representation of over-dilution of the chemical by incorporating too deep (Adapted from Woods, 1997).*




Unfortunately, granular products are not effective treatments for reducing viable eggs inside cysts, but there may be some inhibition of hatch soon after application. Work by Evans & Wright (1982) showed that oxamyl at only 1 microgram ml was enough to inhibit hatch of *G. rostochiensis* and that 4 microgram ml completely inhibited hatch. They also noted that hatching resumed immediately after cysts were removed from oxamyl concentrations. This can be related to the work by Woods (1997) where concentrations of oxamyl in the potato ridge ranged from 1.5 to 4.1 microgram/g soil soon after application, which suggests that hatch inhibition would occur. After 3 weeks however, the concentration could be as low as 0.4 microgram/g soil, which would be insufficient to prevent hatch, or even paralyse juveniles.

The effectiveness of any granular nematicide is directly linked to the accuracy of application and incorporation, but what is less well known is the likelihood of biodegradation of products in the soil that might make them less effective. Osborn (2005) demonstrated that the ecology of different soils can influence the persistence of chemicals. The work showed that some soils have specific soil biota which use the chemicals as a carbon source and thus degrade the chemical more quickly, giving 'enhanced degradation'. The repeated use of products of similar characteristics is also suggested to lead to enhanced degradation by the selection of a soil microflora that metabolizes these compounds. The potential for enhanced degradation of the carbamoyloxime nematicide oxamyl (Vydate® 10G), as well as the organophosphate fosthiazate (Nemathorin®, produced by Syngenta), was investigated in 35 UK agricultural soils under laboratory conditions by Osborn et al. (2010). Rapid degradation was observed in 9 out of 10 soils treated with oxamyl, though whether this was by hydrolysis or microbial action is still debated. None of the fosthiazate-treated soils demonstrated enhanced degradation under these conditions.

There are soil fumigants which can be used to target the nematodes whilst still inside the cyst. All fumigants need good soil structure in order to be effective as the liquid vaporises to become a gas and then diffuses through the soil in all directions but predominantly upwards, especially through the applicator leg. Soils which are warm and dry will allow rapid movement of the gases whilst those that are wet and cold will slow movement and prevent adequate penetration through the soil pores.

Currently the liquid Metam 510 (Certis) which contains 510 g/l metam-sodium is used as a soil-sterilant for a wide range of crops including potatoes. In practice it is used for the management of PCN (Certis, 2015). This product is currently only licensed until 31st December 2017 and as with many chemicals will need re-registration during the intervening years. The product is a liquid which is injected into the soil using specialist machinery. Once released into the soil it decomposes to release methyl-isothiocyanate (MITC) which is toxic to a wide range of fungi, insects, nematodes and weed seeds, it is also phytotoxic so is used well in advance of planting. The chemical directly penetrates the body wall of the nematode, interfering with essential enzymatic, nervous and respiratory systems leading to death normally within several hours. Optimum conditions required for the best control include soil temperatures > 10°C, soil moisture between 40 - 60% of field capacity, free from crop residue and a good soil seal.

There is a granular fumigant called Basamid® (Certis) which contains 97% w/w dazomet which is also an MITC precursor but this is seldom used for PCN control alone due to its much higher cost. The product should be applied in the autumn to soils with temperatures of 8°C - 10°C and rising, and soil moisture should be 60% - 70% of field capacity. However, this product is restricted to use one year in three, so a plan is required for the whole farm. This product is normally reserved for higher value crops.

In very general terms, the effectiveness of chemicals is variable, and depends on many environmental factors, as well as the efficiency of incorporation, so in reality the degree of control can vary up to about 80%. Other plant-parasitic nematodes attack potato in the UK, so chemical control may mask the effects of these species. If there is a move to more sustainable methods of control, then it is possible that control of these other species may require alternative strategies.
All companies provide detailed advice on the use of their products on their websites.

Recent work has examined the potential use of polysulphides in PCN management (Kenyon et al. 2014), which result in a decline in the viability of eggs and consequently a reduced hatch of J2s. Coosemans (2005) reduced PCN populations in glasshouse trials using dimethyl disulphide (DMDS). NEMguard® is a product available from the Certis marketing company which is based on polysulphide technology which is currently cleared for use in carrots and parsnips but which has approval pending for use in potatoes. No data on its effectiveness against PCN has been found for this Reference Guide.

Finally, the practice of prophylactic applications to potato fields, or the selective treatment of fields using GPS to so-called hot-spots of infestation, are not recommended as neither appear to have led to improved control. Once PCN is detected, chemical products, if deemed necessary, should be applied over the whole field to manage PCN at low levels. Using 'spot' techniques to save money on chemical application is false economy in the long run as many foci of infestation are likely not to be detected.

The EU sets rules for the sustainable use of pesticides to reduce the risks and impacts of pesticide use on people's health and the environment (Directive 2009/128/EC). The main actions for sustainable use of pesticides include National Action Plans by EU countries to reduce the risks and impacts of pesticide use, training, the establishment of competent authorities and certification systems, information and awareness raising on acute poisoning incidents and chronic poisoning developments, and inspection of equipment in use. All pesticide application equipment will have to be inspected at least once by 2016 to ensure proper and efficient use of any plant protection product. Many requirements are included in the 'Nematicide Stewardship Programme' (NSP) (Anon, 2015b). The full programme, which will probably evolve, and a list of BASIS-approved advisers specialising in PCN management and Nematicide Stewardship, can be viewed at http://nspstewardship.co.uk. The core current requirements however, that relate in particular to the application of granular nematicides and other products for PCN control, require operators to be suitably trained. Additional requirements that have been developed are:

1) Operators have to be qualified to apply nematicides (NPTC PA4 or PA4G certification);
2) By March 2017 staff applying nematicides must have completed the Industry Stewardship Training module;

3) By March 2017 all applicators must be fitted with a device in cab that allows the operator to shut off nematicide granule flow at least 3 meters from the end of each row.

### *7.7. Biological Control*

> Currently biological control, defined as using parasites or predators of PCN, is not available on a commercial basis, but may be developed in the future. Creating anaerobic conditions, such as flooding, have shown promise abroad, but is considered unlikely to be a practical proposition in the UK. The effects of unintentional flooding on PCN populations have not yet been assessed in the UK!

Biomanagement strategies, using biological control agents such as parasitic fungi, have and are being investigated for their effect on PCN, but have not yet been adapted for large scale use. It is possible that natural controls could be encouraged as part of an integrated control programme, but in the UK climate and soil conditions it is unlikely that they would have a significant effect. A review of the success of microbial control of plant-parasitic nematodes (Dong & Zang, 2006) concluded that whilst various organisms showed promise in laboratory or glasshouse experiments, they showed inconsistent performance in the field, suggesting more research into the complex interactions in the soil environment was required.

Other, naturally-occurring, biological organisms may also have an effect in PCN management. Arbuscular mycorrhizal fungi, according to laboratory tests, may enhance the efficacy of carbamate nematicides by increasing hatch in the first 2-4 weeks after planting, and inhibiting root invasion, but more research about this is required as effects varied for each PCN species, and between potato varieties (Deliopoulos et al. 2011; 2010; 2008; 2007).

There may be scope to investigate UK populations of PCN for natural populations of parasites, but experience to date, especially when considering the commercial production of such organisms, does not provide much data for optimism in UK the climate and soil conditions. An investigation of possible parasites from field populations of cysts in Idaho, USA, produced several species of fungi and bacteria that warranted further investigation (Worapong et al. 2010), and work is being done elsewhere.

#### 7.7.1. *Pochonia chlamydosporia and other fungal parasites*

*P. chlamydosporia* is a fungal parasite of nematode eggs but also a root endophyte known to promote growth of some plants (Zavala-Gonzalez et al. 2015). Manzanilla-Lopez et al. (2013) provide a summary of 30 years of worldwide research on this organism. *P. chlamydosporia* isolated from PCN cysts showed a high variability in pathogenicity in laboratory work done in Portugal, so much research needs to be done if its effects against PCN are to warrant commercial development (dos Santos et al. 2013; dos Santos et al. 2012). Also, studies by Siddiqui et al. (2009), showed that colonisation in soils of different textures (compost, sandy loam and loamy sand) differed greatly, and that most biotypes were more abundant in sterilised soils than non-sterilised soils, indicating some inhibiting factor in the field. Biotypes also had host preferences, with those sourced from *G. pallida* eggs infecting more *G. pallida* eggs than other biotypes. Muthulakshmi et al. (2012) consider the use of this fungus is compatible with other biological control agents and carbofuran. Tobin et al. (2008a) reported the first successful use of *P. chlamydosporia* as a biological control agent against PCN in commercial potato crops, where it was concluded to be as successful as fosthiazate, and though the combined use of the treatments was not significantly better it showed the biological control agent was compatible with the chemical. Commercial fungicides, however, are likely to have a significant effect on this fungus (Tobin et al. 2008b). It is believed that the production of sufficient quantities is the barrier to the commercial use of *P. chlamydosporia*.

Defra funded a research project to determine the impact of nematophagous fungi on the regulation of potato cyst nematode populations, that reported in February, 2006 (HP0115_2229_FRP). Previous to this project, techniques had not been developed to directly monitor nematophagous fungi that attack PCN in the environment. Diagnostic methods were developed that allowed monitoring and enumeration of the fungi for the first time. After the application of these fungi, their survival and ability to control PCN populations can be assessed and rational conclusions made. Baiting allowed an assessment of the pathogenicity to be performed, and, when combined with molecular diagnostics, provided a powerful tool in confirming the biological control ability of a fungal isolate. The optimum inoculation level of the fungi has been demonstrated to be 5000 spores $g^{-1}$ soil, a level that is commercially manageable to produce. The soil type and nematode density are important in the level of infection obtained. The higher the nematode level the greater the infection, thus site-directed application of the fungus to nematode hot spots is an option to be considered. The fungi were able to survive better in peat soil, more as saprophytes than parasites, therefore prolonging its effect. The level of control on the first generation of nema-

todes has been shown to be around 30–50 % with *Plectosphaerella cucumerina* being the better control agent of the three tested. This level of infection would have significant effects on the decline rate of the pest nematode. The fungus can be used in combination with resistant potato varieties to reduce the build up of PCN populations able to increase on these potato varieties, prolonging the use of such control methods. Field trials have shown that the fungi are able to survive the growing season on potatoes after a single application before planting and where *Plectosphaerella cucumerina* was applied significantly increased the level of egg infection. Further work was deemed to be necessary to formulate the biological control agents to increase the level of survival in the soil. From the baiting trials it was demonstrated for the first time that the fungi can remain viable in fallow soil, infecting new cysts once they have been added. This added a new dimension to the work not previously considered. The research assumed that the fungi colonised the rhizosphere and infected emerging females; this is apparent in the work discussed at the beginning of the report. What the baiting trials demonstrated is that the fungi can also infect cysts in the soil and, therefore, need not be added in combination with a potato crop but could be added in rotation with other crops and colonise dormant PCN cysts in the soil and kill the eggs. Previous work has demonstrated that certain crops can boost the level of fungal inoculum added to the soil, and these crops could be added in rotation with the biological control agents in an integrated pest control strategy to reduce the time necessary between susceptible potato crops. For a heavily infested field it is necessary to either apply high levels of nematicides or wait for eight years or longer to grow potatoes again. If application of the biological control agent can reduce the PCN populations significantly then this rotation time and dependency on nematicides could be significantly reduced.

Low temperature scanning electron microscope studies on the interaction of *G. rostochiensis* and *Trichoderma harzianum* have revealed this fungus infected mature potato cyst nematode eggs by penetrating directly the cyst wall or via natural openings such as the mouth (Saifullah & Khan, 2014). A combination of the trap crop *S. sisymbriifolium* and *Trichoderma harzianum* and *Plectosphaerelaa cucumerina* has been investigated by Dandurand et al. (2014b) but further work is required before conclusions can be made.

*Penicillium oxalicum* is a fungus was isolated from the rhizosphere soil of pearl millet. Rhizospheres appear to contain many beneficial, as well as pathological organisms. It has been found to reduce the number of juveniles and cysts of PCN in laboratory conditions

and soil microcosms (small scale investigations) using cv. Desiree (Martinez-Beringola et al. 2013).

*Paecilomyces* sp. were concluded to offer up to 89% control of *G. rostochiensis* in field trials in Mexico (Lopez-Lima et al. 2013). *Pseudomonas fluorescens, Paecilomyces lilacinus* and *Trichoderma viride* were evaluated against *G. pallida* and *G. rostochiensis* in field conditions in India. They were applied in various substrates including neem cake. The effects of Trichoderma are not known, but the Paecilomyces reduced the penetration of roots by J2s by 63-68%, and significantly reduced cyst numbers (Seenivasan et al. 2007). It should be noted that *P. lilacinus* has been implicated in a number of human and animal infections, so proper diagnosis is important (Atkins et al. 2005).

Fungal parasites of *G. rostochiensis* have been investigated in Indonesia, where several fungal isolates of PCN were isolated. (Indarti et al. 2014; 2010). In two experiments thought to have been done in Egypt, parasitism of PCN eggs was significantly affected by the organic amendments also applied, with higher parasitism occurring with maize straw. There was a difference between the effect of fungal species, with *Pochonia chlamydosporia* 392 having the greatest effect (albeit low at around 7%), not only on PCN but also on root-knot nematodes (Uambano & Kerry, 2007).

*7.7.2. Bacterial parasites*

Many bacterial parasites exist in the rhizosphere of plants, and some have been shown to be antagonistic to nematodes, and there have been investigations which showed a suppressive impact of some rhizobacteria which are components of commercial microbiological manures (Oro et al. 2009). Some, such as *Bacillus* spp., produce a chitinase that could degrade the inner layer of PCN eggs (Margino et al. 2012). There have been glasshouse trials investigating the use of *Pseudomonas* spp. and *Bacillus* spp. for the biocontrol of the potato cyst nematode *Globodera rostochiensis (*Trifonova et al. 2014; Nikpay & Khodakaramian, 2013 respectively). The highest mortality rates observed were around 42%. Laboratory experiments using *P. oryzihabitans* against *G. pallida* also inhibited the behaviour of the juveniles and led to 100% mortality. Thus the use of commercial products requires greater scrutiny.

*7.7.3. Manipulating PCN/plant interactions*

As knowledge is gained about the interactions between PCN and its hosts, so this might be transformed into innovative methods of control, but much further research is needed. There are two categories of factors that effect PCN hatch, those within the cyst that stimulate hatch and would be used in the absence of a commercial potato crop, and those

that inhibit hatch. Research has looked at both artificial and plant-derived agents, with some results indicating that the approach has some prospect. However, there are production and storage issues for use at field scale (Jones et al. 1998). Tanino et al. (2011) synthesised solanoeclepin A, stated to be the key hatch-stimulating substance for PCN, but no records were found of a commercially-available product.

*7.7.4. Control using miscellaneous plant extracts, agricultural residues and other soil amendments*

The effect of numerous other plant extracts, adjuvants and organic amendments has been and continues to be studied. Overall, it is essential that initial glasshouse trials are followed up by field trials to prove the effectiveness of such compounds in an environment where we are still making new discoveries.

The use of industrial or agricultural wastes to reduce PCN does not appear to have been investigated in the UK, but is underway in some countries such as Portugal, where the possible effects of agricultural waste or by-products on PCN and other plant-parasitic nematodes is being investigated. In laboratory conditions, liquid swine manure controlled PCN, probably due to the nematicidal action of fatty acids contained therein, (Lopez-Robles et al. 2013), but further field investigations are required.

Waste from the logging and timber industry (freshly-crushed conifer bark and sodium lignosulphonates, products of the pulp and paper industries) delayed the onset of hatching and decreased the total juvenile abundance and the viability of eggs and juveniles inside cysts (Matveeva et al. 2010).

The effect of soil compost treatments on PCN has been studied in pots (Renco et al. 2011; 2007; D'Addabbo et al. 2008). Mixtures containing by-products from penicillin production (mycelium), cow manure, grape pomace, grass, horse manure, leaves, lettuce residues, municipal green residues, olive pomace, pig manure, poultry manure, sawdust, sewage sludge, straw, sugar beet pomace, tree branches, urea, vermicompost from medicinal

plant wastes and vermicompost from cattle manure all produced a significant reduction in the number of eggs, juveniles and cysts, with the suppressive nematode effect increasing in relation to the NH(4)(+) content and compost rate.

Chitosan (a linear polysaccharide composed of randomly distributed β--linked D-glucosamine and N-acetyl-D-glucosamine and made by treating shrimp and other crustacean shells with the alkali sodium hydroxide) has been investigated for control of PCN, and found to be effective in pot experiments such as the work by Lopez & Aymerich (2005).

Garlic extract products have been patented (Bahiri & Elliot, 2014). An aqueous garlic extract (salicylaldehyde) was examined for its effectiveness against *G. pallida* in glasshouse conditions and found to be disruptive against J2s. Early laboratory work had shown salicylaldehyde was more toxic than the garlic extract, and seemed to stimulate the hatch of *G. pallida* (Danquah et al. 2013; 2011). High concentrations of a natural biopesticide, dimethyl disulphide, obtained from *Allium* spp., significantly reduced the development of PCN in glasshouse trials (Faruk et al. 2010).

The nematicidal potential of *Artemisia annual*, or sweet wormwood, which already has medicinal uses, has been investigated in laboratory conditions by D'Addabbo et al. (2014) for use against *G. rostochiensis*, and found to be highly effective against J2s, although it did not prevent egg hatch, and they recommend more trials in the field.

Renco et al. (2012) investigated chestnut tannin solutions on *G. rostochiensis* in laboratory assays, and found that egg viability was reduced by 56-87% and reduced the numbers of cysts, eggs, juveniles and reproduction rate in soil.

Jasmonate plant hormones activate genes involved in pathogen control. Their use as biological control agents has been investigated in laboratory experiments and found to reduce infestation levels of *G. pallida* by 63%, as well as affecting nematode development inside the roots (Pankaj et al. 2013). They suggest the use of seed treatments containing these chemicals to protect potatoes against PCN.

A neem-based product was investigated in a glasshouse study; potato plant growth and yield was improved and nematode reproduction was reduced by 59.8 to 71% (Trifonova et al. 2012; Trifonova & Atanasova, 2011).

*7.7.5. Inundation (flooding) and other anaerobic soil disinfestations*

Inundation, or flooding, creates anaerobic conditions that has been shown to reduce root-knot nematodes in Europe (Collange et al. 2011), and it is has been recorded as a method for the control of PCN by Runia et al. (2014a). After 16 weeks of inundation, 99.9% of the contents of artificially applied *G. pallida* cysts were eliminated, and apparently the method has been adopted in The Netherlands by seed potato growers. They concluded the inundation was economically feasible. However, in the UK, it is likely that flooding would be costly, damage soil structure and not be a feasible option; to date, PCN has been spread by flooding (and irrigation), not controlled by it.

Other anaerobic soil disinfestations include the wetting of soil, incorporation of fresh organic matter and covering with an airtight plastic foil for several weeks (Runia et al. 2014b). Studies involving the use of a defined agricultural product, 'Herbie', recorded the decline of eggs in the cysts to levels less than 0.5% at the end of the experiment. The rate of egg inactivation depended significantly on soil type, although texture (sand vs. clay) appeared unimportant.

***Chapter 8 Decision pathways***

The use of scenarios to illustrate decision-making processes in PCN management is fraught with problems as each farm, and even each field, will pose different problems for each market sector. It should be apparent that the factors controlling the populations of PCN are complex and inter-related, including the species present, population levels at planting, weather, soil type, the resistance and tolerance of the potato varieties used, length of rotation, and the presence of volunteer potato plants, to name just a few. We have attempted to solve this problem with generic flow diagrams showing decision pathways, following the principles of PCN management as set out in this Reference Guide.

The Project Consortium examined the feasibility of producing scenarios for PCN management, but concluded that it was difficult to be prescriptive for every field situation, or for every market sector. It is also recognised that farmers may rent out land for potato cropping, or may indeed use rented land for potato cropping. In both scenarios, there may be limited management options. However, if PCN is to be managed effectively, all stakeholders should come to an agreement concerning the measures to be taken in any particular field to ensure the long-term sustainability of that field. The history of PCN species, their population levels and cropping history are amongst the factors that should be recorded. Two generic flow diagrams to illustrate decision pathways have been devised to assist in this process (Figs. 16 and 17) and users are advised to consult relevant Chapters in this Reference Guide for more detailed advice.

Fig.16. Summary of PCN Management - Principles (in an accompanying file)

Fig.17. Summary of PCN Management - Anticipated Outcomes of Variety Choice (in an accompanying file)

***Chapter 9 Concurrent Projects and Future Research Needs***

Inevitably, the production of such a detailed review and Reference Guide does reveal gaps in our knowledge, and progress with on-going research will undoubtedly require that this Reference Guide is updated to remain relevant. Thus the following recommendations should be noted:

- The results of current AHDB and Defra projects/consultations which may have an influence on the PCN Management Advice given in this Reference Guide need to be considered, i.e.
  - the Review of PCN processing and identification procedures in UK commercial laboratories;
  - Sampling procedures;
  - Distribution of PCN species in England, Wales and Scotland
  - Investigation into PCN trap crops
- If possible, expedite the integration of data from the European CPD into the (GB) PVD so growers have a one-stop shop for variety details. The (GB) PVD is a good reference tool but needs to be updated regularly to reflect current market trends.
- Despite the wealth of literature on PCN, there remains more work to be done to provide field evidence in UK conditions for guidance on many aspects, such as fundamental knowledge on the distribution of species, the pathogenicity of populations, interactions between field populations of PCN and new varieties (with new traits) in several respects, such as life cycle, and improved estimates of risk of damage to the potato crop.
- The financial costs of PCN infestations need to be more widely known as an incentive to promote long term management plans.
- Likewise, there is a need to assess the financial benefits of all components of a PCN management programme, including cultural methods, such as extending the length of rotation and hygiene, as well as chemical control. Thus the financial benefits of using, say, extended rotations rather than or as well as chemical control to reduce PCN populations could be realised, as well as the financial benefits of using resistant varieties.
- Kasia Dybal-Lima at Harper Adams University is working on a new survey of PCN species distribution in England and Wales, and samples will be subject to the metagenomic approach as has been very recently used on Scottish samples to determine the diversity and distribution of PCN at the country, field and individual level. This research should be added to this Reference Guide as soon as it becomes available.
- Consideration should be given to a ‘clean machine’ charter, perhaps supported by a survey of soil sampled and processed from farm machinery and vehicles transporting

potatoes from farm to processing sites and the financial benefits for giving hygiene greater importance.

- There is a need to promote growers' own management plans that are successful in controlling PCN, to encourage and support others, and the action being promoted in these guidelines.
- The NSP should review its guidance in the light of recommendations made in this Reference Guide.
- The PCN Calculator requires greater scrutiny if it is to be used to assist in PCN Management Programmes. Notes on the AHDB website stated this was not the original intention, because of a paucity of data. The authors of this Reference Guide failed to secure what they regarded as reliable results from several scenarios. More resource should be invested to ensure this facility is updated and reinforced with more field data to provide realistic outcomes.
- Lastly, but not least, the information in this Reference Guide is likely to need updating at least on a yearly basis, and resource should be provided to do this to maintain this Guide as a true reference point for industry.

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**Glossary**

| Term used | Defined |
|---|---|
| abiotic | not derived from living organisms |
| allelopathy | the chemical inhibition of one plant (or other organism) by another, due to the release into the environment of substances acting as germination or growth inhibitors |
| anthropogenic | of environmental pollution and pollutants originating in human activity |
| auxin | a plant hormone which causes the elongation of cells in shoots and is involved in regulating plant growth |
| biocide | a poisonous substance, especially a pesticide |
| biofumigant | a process that occurs when volatile compounds with pesticidal properties are released during decomposition of plant materials or animal products |
| biotrophic | entirely dependent upon another living organism as a source of nutrients |
| coevolutionary | process by which two species influence each other during evolution. For example, an insect may evolve specialized parts that allow it to feed on a specific flower, whereas the flower evolves to facilitate pollination by that particular insect. |
| conducting tissue | Vascular tissue is a complex conducting tissue, formed of more than one cell type, found in vascular plants. The primary components of vascular tissue are the xylem and phloem. These two tissues transport fluid and nutrients internally |
| Cortex | an outer layer of tissue immediately below the epidermis of a stem or root |
| cytokinins | a class of plant hormones synthethised mainly in root tips that promote cell division and growth and delay the senescence of leaves |
| diapause | a period of suspended development, especially during unfavourable environmental conditions |
| DNA | DeoxyriboNucleicAcid, a self-replicating material which is the main component of chromosomes. It is the carrier of genetic information |
| effector | a molecule that binds to a protein and affects the functions of that protein |
| focus (plural foci) | a centre of interest, attention or activity |
| gene pool | the stock of different genes in an interbreeding population |
| genome | the complete set of chromosomes in a cell or an organism |
| genome sequence | the order of DNA nucleotides, or bases, in a genome that makes up an organism's DNA |
| genotype | the genetic constitution of an individual organism |
| glucosinolates | natural components of many pungent plants, such as mustard |
| glycoside | a compound formed from a simple sugar and another compound by replacement of a hydroxyl group in the sugar molecule. Many drugs and poisons derived from plants are glycosides. |

| Term used | Defined |
|---|---|
| half-life | the time required for any specified property (e.g. the concentration of a substance) to decrease by half |
| hatching factor stimulants | part of the potato root leachate identified as having a role in hatching |
| isothiocyanates | a pungent and irritating sulphur analog of an isocyanate |
| mitochondrion | a structure located in the cytoplasm, outside the nucleus of a cell |
| mitochondrial genes | genetic material found in mitochondria |
| molecular mimicry | where a foreign antigen or invader shares sequence or structural similarities with host antigens |
| molecular tools | biological tools or assays used in the analysis and diagnosis of organisms |
| morphological | dealing with the form and structure of organisms |
| necrotic | used to describe the death of cells through injury or disease |
| nematicide | a substance to kill nematodes |
| nematistat | a substance that prohibits movement and feeding; death can occur if feeding is prevented long enough to cause starvation, but nematodes can recover and resume normal activity if the presence of the substance subsides |
| pathogenicity | the ability of an organism to harm the host |
| pathotype | a variant of a micro-organism, distinguished from other members of the species by its molecular markers and virulence |
| perivitelline fluid | fluid found in the space between the fertilisation membrane and the ovum after the entry of sperm into the egg |
| phenotype | the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment |
| pseudo-resistance | apparent resistance in a potentially susceptible host resulting from chance, from non-inheritable traits or from environmental conditions |
| quantitative trait locus (plural loci) | a section of DNA (the locus) that correlates with variation in a phenotype (the quantitative trait) and which is typically linked to, or contains the genes that control that phenotype |
| quiescent | in a state or period of inactivity or dormancy |
| R gene | Resistance gene |
| rDNA | recombinant DNA, the combination of two DNA strands that are constructed artificially |
| regressions | in statistics, a measure of the relation between the mean variable of one value and corresponding values of other variables |
| rhizosphere | the region of soil in the vicinity of plant roots in which the chemistry and microbiology is influenced by their growth, respiration and nutrient exchange |

| Term used | Defined |
|---|---|
| RNA | ribonucleic acid, an acid present in all living cells, whose principal role is to act as a messenger carrying instructions from DNA for controlling the synthesis of proteins |
| root diffusate | solution produced by potato roots |
| rRNA | ribosomal RNA, a molecular component of a ribosome, the cells' essential protein factory |
| saponins | a compound in the class of steroid and terpenoid glycosides which foam when shaken with water, e.g. as used in detergents |
| solanacoeous | relating to or denoting plants of the nightshade family (Solanaceae) |
| statistically valid | the extent to which a concept, conclusion or measurement is well-founded and corresponds accurately to the real world |
| supercoding | a process that defines functionality |
| syncytium (plural syncytia) | a single cell or cytoplasmic mass containing several nuclei |
| synonym | a taxonomic name which has the same application as another, especially one which has been superseded and is no longer valid |
| synonymisation | to demonstrate a name to be a synonym |
| synthesis | the production of chemical compounds from simpler materials |
| transcriptomic | derived from transcriptome, the full range of messenger RNA, or mRNA, molecules expressed by an organism |
| trap crop | in nematology, a crop planted to attract nematodes, especially one with which they fail to survive or reproduce |
| vermiform | resembling or having the form of a worm |
| viability | the ability of an organism to maintain itself or recover its potentialities |
| Viable cyst | capable of living or surviving successfully |
| virulence | in nematology, a harmful quality possessed by a nematode that can affect a plant |

***Disclaimer for Potato Cyst Nematodes (PCN) - Their Characteristics and Guide to Management***

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