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Report for Winetech:

Literature Study on Control of Nematodes in Irrigation Water

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1. INTRODUCTION

The presence of free-living nematodes in drinking water was already known early in the previous century (Tombes *et al*., 1978, 1979). Godfrey (1923) was the first to point out the possibility of plant parasitic nematodes (PPN) being dispersed by irrigation water. Pioneering research by Faulkner & Bolander (1966, 1970a, b) showed 10-20% of the total nematode population in a main irrigation canal in Washington to be plant parasitic, and thereby demonstrated the potential for spreading PPN via irrigation water.

Rivers and irrigation water are also important sources of nematode distribution in the Western Cape, as have been shown by surveys along the Berg and Breede rivers (Barbercheck *et al.,* 1985; Van Reenen & Heyns, 1986). Subsequently VinPro-Worcester identified nematodes in irrigation water as a problem.

Irrigating directly from such infested sources poses a serious nematode threat to vineyards and nurseries. Also, *Xiphinema index*, the vector of grapevine fanleaf virus is continuing to spread to new vine growing areas. One of the possible causes of this spread is infested irrigation water.

During the 1970's and 1980's work was done by the then Dept. of Agriculture on the distribution and possible control of nematodes in irrigation water. Since 1992 no further research has been done. The objective of this study was to consolidate available information, and to discuss possible future strategies to prevent or control PPN contamination of irrigation water in South Africa, with special reference to vine nurseries. Examining methods to prevent the degradation of irrigation water and preserving it as a vital source for agricultural use was a paramount objective of this study.

2. PLANT PARASITIC NEMATODES REPORTED FROM IRRIGATION WATER

All of the major economically important PPN genera have been found in surveys of irrigation canals, rivers, dams, runoff from agricultural fields, municipal, and drainage water from soilless hydroponic systems world-wide (Table 1). The economically most important PPN for the South African vine growers, including nurseries, are the following:

Meloidogyne spp. (root-knot nematode)

Xiphinema index (dagger nematode) (vector of grapevine fanleaf virus GFLV)

Mesocriconema xenoplax (ring nematode)

Pratylenchus spp. (lesion nematode)

Thomason & Faulkner (1975) assumed that nematode virus vectors may be spread by means of irrigation water. In South Africa, *X. index* and GFLV is a major problem in the grapevine industry. *Xiphinema* spp. have been reported from irrigation water, mostly from irrigation canals (Faulkner & Bolander, 1970a; Waliullah, 1984, 1989; Roccuzzo & Ciancio, 1991), but also in runoff water (Heald & Johnson, 1969), and rivers and dams (Smith & Van Mieghem, 1983a). *Xiphinema index,* specifically, was reported from irrigation canals in India and Italy (Waliullah, 1989; Roccuzzo & Ciancio, 1991).

Research done in Senegal showed that runoff water can play a major role in the passive transport of PPN (Cadet & Albergel, 1999; Cadet *et al*., 2002). It was estimated that the total run-off during the rainy season was 6 000 m^3 water and 18,6 t soil. This included 280 x 10^6 nematodes, of which 127 x 10^6 were PPN (Cadet & Albergel, 1999). Data suggest that transport by runoff water and water-related behaviour of nematodes is not only dependent on host-parasite relationships, but also on the survival strategies which could promote certain nematode species. Transport of PPN can add to the potential negative impact of runoff water (Cadet *et al*., 2002).

Table 1 Plant parasitic nematode species recovered from irrigation water (*S.A. references in bold*)

Species	Water source	Locality	Reference
Aphelenchoides compositola	Canal	India (Kashmir Valley)	Waliullah, 1989
A. ritzemabosi	Canal	India (Kashmir Valley)	Waliullah, 1989
Aphelenchoides spp.	Canal	India (Upper Ganges Channel); USA (Nebraska)	Steadman *et al*., 1975; Waliullah, 1984;
***Criconema* sp**	**Dam, river,**	**South Africa (Western Cape)**	**Smith & Van Mieghem, 1983b**
Criconemella curvata	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999
Criconemoides siddiqi	Canal	India (Kashmir Valley)	Waliullah, 1989
Criconemoides spp.	Runoff	Australia (Victoria); USA (Georgia)	Meagher, 1967; Heald & Johnson, 1969
Ditylenchus brassicae	Canal	India (Kashmir Valley)	Waliullah, 1989
D. dipsaci	Canal, runoff	USA (Utah; Washington)	Faulkner & Bolander, 1970a
D. myceliophagus	Canal	India (Kashmir Valley)	Waliullah, 1989
D. nanus	Canal	India (Kashmir Valley)	Waliullah, 1989
***Ditylenchus* spp**.	Canal**, dam, river**	**South Africa (Western Cape** ; USA (Washington, Nebraska)	**Smith &VVan Mieghem, 1983b**; Faulkner & Bolander, 1966, 1970a; Steadman *et al*., 1975
Gracilacus parvula	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999
Heterodera spp.	Canal, runoff	USA (Washington, Nebraska))	Faulkner & Bolander, 1966, 1970a, b; Steadman *et al*., 1975
Helicotylenchus abunaamai	Canal	India (Kashmir Valley)	Waliullah, 1989
H. crenacauda	Canal	India (Kashmir Valley)	Waliullah, 1989
H. dihystera	Canal, runoff	India (Kashmir Valley); Senegal (Sudano-Sahelian area)	Waliullah, 1989; Cadet & Albergel, 1999; Cadet *et al*., 2002
H. indicus	Canal	India (Kashmir Valley)	Waliullah, 1989
H. insignus	Canal	India (Kashmir Valley)	Waliullah, 1989

Species	Water source	Locality	Reference
H. hazratbalensis	Canal	India (Kashmir Valley)	Waliullah, 1989
H. kashmiriensis	Canal	India (Kashmir Valley)	Waliullah, 1989
H. mucronatus	Canal	India (Kashmir Valley)	Waliullah, 1989
H. vulgaris	Canal	Southern Italy	Roccuzzo & Ciancio, 1991
***Helicotylenchus* spp**.	Canal, **dam**, **river,** runoff	India (Upper Ganges Canal); **South Africa (Western Cape)** ; USA (Georgia)	Waliullah, 1984; **Smith & Van Mieghem, 1983a, b**; Heald & Johnson, 1969
Hemicriconemoides	Canal	India (Upper Ganges Canals)	Waliullah, 1984
Hemicycliophora indica	Canal	India (Kashmir Valley)	Waliullah, 1989
***Hemicycliophora* spp**.	Canal, dam, **municipal water**, river, runoff	Australia (Victoria); **South Africa (Western Cape);** USA (Washington)	Meagher, 1967; Faulkner & Bolander, 1966; **Smith & Van Mieghem, 1983a, b**
Hirschmanniella mucronata	Canal	India (Kashmir Valley)	Waliullah, 1989
H. oryzae	Canal	India (Kashmir Valley)	Waliullah, 1989
Hoplolaiminae	**Dam, municipal water, river**	**South Africa (Western Cape)**	**Smith & Van Mieghem, 1983a, b**
Hoplolaimus spp.	Canal, dam, runoff	India (Kashmir Valley); USA (Georgia)	Waliullah, 1989; Shokes & McCarter, 1979;
Longidorus reneyii	Canal	India (Kashmir Valley)	Waliullah, 1989
L. Brevis	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999
***Longidorus* spp**.	Canal**, dam**, **river**	India (Upper Ganges Canals)**, South Africa (Western Cape)**	Waliullah, 1984; **Smith & Van Mieghem, 1983b;**
Macroposthonia sp	**Dam**, **river**	**South Africa (Western Cape)**; USA (Georgia)	**Smith & Van Mieghem, 1983b**; Shokes & McCarter, 1979
Meloidogyne incognita	Drain water	Belgium (soilless culture)	Moens & Hendrickx, 1990
M. arenaria	Drain water	Italy (soilless culture)	D'Errico & Ingenito, 2003
***Meloidogyne* spp.**	Canal, **dam**, **municipal water, river**, runoff	Australia (Victoria); **South Africa (Mpumalanga; Western Cape Province);** USA (Washington, Georgia)	Meagher,1967; **Grech *et al*., 1989**; **Smith & Van Mieghem, 1983a, b;** Faulkner & Bolander, 1966, 1970a; b; Heald & Johnson, 1969
Paralongidorus sali	Canal	India (Kashmir Valley)	Waliullah, 1989
***Paratylenchus* spp.**	Canal, **dam, municipal water, river,** runoff	Australia (Victoria); India (Kashmir Valley); Senegal (Sudano-Sahelian area); **South Africa (Stellenbosch, Western Cape)**; USA (Georgia, Nebraska, Washington)	Meagher, 1967; Waliullah, 1989; Cadet & Albergel, 1999; **Smith & Van Mieghem, 1983a, b**; Heald & Johnson, 1969; Faulkner & Bolander, 1966, 1970a, b
Pratylenchus neocapitatus	Canal	India (Kashmir Valley)	Waluillah, 1989
P. pseudopratensis	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999; Cadet *et al*., 2002
P. similis	Canal	India (Kashmir Valley)	Waliullah, 1989
P. zeae	Canal	India (Kashmir Valley)	Waliullah, 1989
***Pratylenchus* spp**.	Canal, **dam**, **river**, runoff	Australia (Victoria); India (Kashmir Valley); **South Africa (Western Cape, Mpumalanga)**; USA (Nebraska; Washington)	Meagher, 1967; Waliullah, 1984, 1989**; Smith & Van Mieghem, 1983b; Grech *et al*., 1989;** Faulkner & Bolander, 1966, 1970a, b; Heald & Johnson, 1969; Steadman *et al*., 1975
Radopholus similis	Drain water	Belgium (Soilless culture)	Moens & Hendrickx, 1989

Species	Water source	Locality	Reference
Rotylenchus spp.	Canal	Upper Ganges Canals, India	Waliullah, 1984
Ring nematodes	Canal**, dam**, **river,** runoff	Australia (Victoria); India (Kashmir Valley); Senegal (Sudano-Sahelian area)**; South Africa (Western Cape)**; USA (Georgia)	Meagher, 1967; Waliullah, 1989; Cadet & Albergel, 1999; **Smith & Van Mieghem, 1983b;** Heald & Johnson, 1969
Scutellonema cavenessi	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999; Cadet *et al*., 2002
Scutellonema spp.	Canal	India (Kashmir Valley)	Waliullah, 1989
***Trichodorus* spp**.	Canal, **dam, municipal water, river**, runoff	India (Upper Ganges Canal, Kashmir Valley); **South Africa (Western Cape);** USA (Washington, Georgia)	Waliullah, 1984; 1989; **Smith & Van Mieghem, 1983a, b**; Faulkner & Bolander, 1966; Heald & Johnson, 1969
Tylenchulus semipenetrans	**River**	**South Africa (Mpumalanga)**	**Cohn, 1976a, b; Grech *et al*., 1989; Grech & Rijkenberg, 1992**
Tylenchorhynchus baki	Canal	India (Kashmir Valley)	Waliullah, 1989
T. brassicae	Canal	India (Kashmir Valley)	Waliullah, 1989
T. gladiolus	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999; Cadet *et al*., 2002
T. kashmiriensis	Canal	India (Kashmir Valley)	Waliullah, 1989
T. mashhoodi	Canal, runoff	India (Kashmir Valley)	Waliullah, 1989
T. sulcatus	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999
T. ventralis	Runoff	Senegal (Sudano-Sahelian area)	Cadet & Albergel, 1999
***Tylenchorhynchus* spp**.	Canal, **dam**, **river**	India (Upper Ganges Canals); **South Africa (Western Cape Province)**; USA (Georgia, Washington)	Waliullah, 1984; **Smith & Van Mieghem, 1983b**; Heald & Johnson, 1969; Shokes & McCarter, 1979; Faulkner & Bolander, 1966, 1970a, b
Xiphinema americanum	Canal	India (Kashmir Valley)	Waliullah, 1989
X. basiri	Canal	India (Kashmir Valley)	Waliullah, 1989
X. index	Canal	India (Kashmir Valley); Southern Italy	Waliullah, 1989; Roccuzzo & Ciancio, 1991
Xiphinema spp.	Canal, **dam**, **river**, runoff	India (Upper Ganges Canals), **South Africa (Western Cape**); USA (Georgia, Washington)	Waliullah, 1984**; Smith & Van Mieghem, 1983b**; Heald & Johnson, 1969; Faulkner & Bolander, 1970a

3. SOURCES OF CONTAMINATION OF IRRIGATION WATER WITH PLANT PARASITIC NEMATODES

Although PPN live in the water film around soil particles, they are not adapted to an aquatic lifestyle. As they are not natural inhabitants of water, the origin of a water source will most probably be free of PPN. However, they can enter the irrigation system in several ways along its distribution path. Also, nematodes not endemic to a specific area may be brought into a production area with irrigation water from a river or canal. *Xiphinema index,* for example, is not endemic to South Africa, and was most probably imported many years ago in soil clinging to grapevine roots.

Intensified farming is itself the unavoidable main source of contamination of irrigation water with PPN. Producers should be aware that irrigation water can be contaminated, and production methods should be adapted to prevent or control infestation.

Possible sources of nematode-contamination in irrigation water are:

A. Wells and boreholes

Water from a deep well in Washington state, USA, was compared to water from a major irrigation canal over a period of three years by irrigating susceptible host plants growing in methyl bromide fumigated screenhouse ground beds. Beds became heavily infested with nematodes from canal irrigation water, whereas no nematodes were found in the beds irrigated with water from the well (Faulkner & Bolander, 1970b).

Water from wells and boreholes can generally be regarded as free from PPN. However, if the well or borehole is not cased or if the head is not sealed near the surface, runoff carrying sediment contaminated with PPN can enter the well and contaminate the water (Hong & Moorman, 2005).

B. Collected rainwater

Nematodes can be transported in dust and contaminate rainfall. Rainwater collected in cisterns filled by rainfall from a roof cannot automatically be regarded as free from PPN (Baujard & Martiny, 1994). Research carried out in the peanut cropping area of Senegal showed that PPN are transported by wind in an anhydrobiotic condition as eggs or juveniles. The nematodes recovered from dust included species of *Tylenchorhynchus*, *Hoplolaimus*, *Helicotylenchus* and *Paratylenchus*. Contact with sand particles and high temperatures on the soil surface during transport by wind, did not affect the potential of these species to reproduce on host plants (Baujard & Martiny, 1994).

C. Ponds, lakes and dams

Ponds, lakes and dams may be infected with PPN. These nematodes can originate from the underlying and surrounding soil or plant debris or from runoff water from neighbouring agricultural land.

Six surface irrigation ponds in southern Georgia, USA, were surveyed for plant pathogens by taking samples from the top, middle and bottom of the pond (Shokes & McCarter, 1979). Low populations of PPN (*Hoplolaimus* sp., *Tylenchorhynchus* sp. and *Macroposthonia* sp.) were only found in the bottom sediment of the ponds. Water from these ponds was used to irrigate fumigated soil from which vegetable and ornamental transplants were shipped to other areas of the USA. Recovery of a few *Hoplolaimus* specimens from an

irrigation line in a grower's field proved that nematodes are disseminated with the irrigation water from these ponds (Shokes & McCarter, 1979).

D. Rivers

Nematodes may enter river water from the roots of infected plants growing on river banks. Thomason & Van Gundy (1961) detected two *Meloidogyne* species in the roots of weeds growing on the banks of the Colorado River in immediate contact with the water.

Cohn (1976a, b) referred to the quality of irrigation water available from the Crocodile River, Mpumalanga, (formerly Eastern Transvaal) as "soup" and not water. He estimated that the flow of citrus nematode larvae (*Tylenchulus semipenetrans*) in the river was 7×10^9 per hour during the summer months.

Seven representative sources of irrigation water in the Western Cape were monitored at regular intervals for the occurrence of PPN, especially potential virus vectors. Plant parasitic nematode numbers in river water were found to be considerably higher than from dams (Smith & Van Mieghem, 1983a).

The spread of *Xiphinema index* from the Robertson and Bonnievale areas along the Breede River is most probably the consequence of irrigating directly from the river (Barbercheck *et al*., 1985).

E. Municipal water

Smith & Van Mieghem (1983a) were the first to report free-living and PPN from Stellenbosch municipal tap water. They found that this water was derived from two sources. During the period April-January, depending on the rainfall, water from the Eerste River is diverted directly into the municipal distribution network without filtration. The standard chlorination treatment applied is non-toxic to nematodes. During the same period river water from the same source is also pumped into the municipal storage reservoirs to augment water supplies for the rest of the year. Before distribution the water from the reservoirs is passed through a sand filtration system and chlorinated. Nematodes were only found in water that did not pass through the sand filters, but was directly pumped into the system and their occurrence was also seasonal, with higher numbers during the rainy winter months. This water was obtained directly from the Eerste River, indicating that the source of the nematodes was the river.

F. Runoff water

In Georgia, USA, container-grown woody ornamentals were irrigated from a pond refilled by runoff water from the containers. When nematode counts were made of the runoff water returning to the pond, it was found to be infested with *Criconemoides*, *Helicotylenchus*, *Pratylenchus*, *Meloidogyne*, *Tylenchorhynchus*, *Hoplolaimus* and *Trichodorus* (Heald & Johnson, 1969).

Faulkner & Bolander (1970a) concluded from their sampling of the Columbia Basin in Washington state, USA, that the PPN entered the West Canal via the runoff water from irrigated fields as samples from the upper area of the basin without irrigated fields next to its banks failed to yield PPN during a three year testing period.

In the Sudano-Sahelian area in Senegal, a study was done to quantify the role of runoff water in nematode distribution (Cadet & Albergel, 1999). *Gracilacus parvula, Helicotylenchus dihystera, Pratylenchus pseudopratensis, Scutellonema caveness, Tylenchorhynchus gladiolus* and *T. mashoodi* were recovered from the runoff water.

Studies on the impact of runoff on erosion and transport of nematodes were undertaken, using simulated rainfall in Senegal at the end of the dry season (Cadet *et al*., 2002). Plant parasitic species were found in much lower numbers than expected from the volume of soil recovered, whereas the reverse was true for the free-living nematodes. *Scutellonema cavenessi* was found in much higher proportions in runoff water than in soil. The reverse was true for *Tylenchorhynchus gladiolus*, despite its abundance in the soil. The water required to move nematodes was almost four times less than required to move soil particles (Cadet *et al*., 2002). Villenave *et al*. (2003) used the same method with simulated rainfall to study the transport of free-living nematodes by runoff water in the Sudano-Sahelian area in Senegal. They found that all the nematode taxa present in the soil were also present in the runoff water, but in lower numbers. Their abundance was influenced by their occurrence in the first few millimetres of soil and by the shape of the specific nematode. It was concluded that the rapid infestation of soil by PPN after fallow to cultivation of land, or from annual crops to tree plantations, was because of nematodes being transported by the rain runoff water.

G. Irrigation canals

Faulkner & Bolander (1966) found large numbers of nematodes in irrigation canals in Washington state, USA. Nematode distribution in the canals was found to be random in any portion of the canals and an estimated 16×10^6 nematodes per day were carried past a given point. They also calculated that each time 0,4 ha (1 acre) of land was irrigated it can receive between $4\text{-}10 \times 10^6$ PPN (Faulkner & Bolander, 1966).

Waliullah (1989) found between 20-115 nematodes per 50 litres of water in upland canals and 65-290 nematodes in lowland canals. It was estimated from flow data that in 24 hours between 90×10^6 to 470×10^6 nematodes passed through the main canals and 15×10^4 to 24×10^5 nematodes through the side canals.

H. Drainage water in soilless culture

Changing from a soil to a soilless culture system for crops in glasshouses did not eliminate PPN. Nematodes are not expected to be present in soilless cultures and are therefore easily overlooked until an infestation occurs (Schnitzler, 2004; Combrink, 2005; Hallmann *et al*., 2005). The most common source of nematode infestations in soilless culture systems is either infested planting material or contminated water. Infected plants, by releasing nematodes into the circulated nutrient solution of soilless hydroponic systems constitute the primary infection source in hydroponic-type systems (Hallmann *et al*., 2005).

Closed soilless hydroponic systems are also an ideal experimental setup for testing methods for eliminating PPN from irrigation water (Moens & Hendrikx, 1989; Moens *et al*., 1991; Runia, 1995; Runia & Amsing, 1996). As large volumes of crops are grown in closed hydroponic systems in the Netherlands, research over the last 15 years focused on the elimination of PPN from the nutrient drain water in such systems.

Moens & Hendrickx (1990) found PPN in the nutrient solution of hydroponic-like systems used for growing ornamental pot plants. They demonstrated that the second stage juveniles of *Meloidogyne incognita* present in drain water can re-infect the roots of tomato plants. Nematode infestations in soilless culture have also been observed in Hawaii and Italy (Wang *et al*., 1997; D'Errico & Ingenito, 2003).

4. DETECTION AND MONITORING METHODS FOR PLANT PARASITIC NEMATODES IN IRRIGATION WATER

A. Sampling methodology

There is enough evidence in the literature of the occurrence and dissemination of PPN by most types of irrigation water. It is therefore important to be able to detect and monitor for their presence in a specific production setup. Because of the cost involved in controlling nematodes, techniques are needed to determine their presence in irrigation water.

Sampling irrigation water for nematodes can be very simple, such as just dipping a container in the water or more elaborate by using a custom-made apparatus. The water is then poured through sieves with different pore sizes to concentrate the nematodes. The choice of pore size has two objectives: to prevent clogging, and to extract the largest variety of PPN present in a sample.

Decisions on the number and volume of samples depend on the detection threshold which can be determined by trial and error (Hong & Moorman, 2005). Faulkner & Bolander (1966) determined the optimum size of sampling by taking five samples each of 4, 8, 12, 19 and 27 litres of water from an irrigation canal. The

number of nematodes from each volume was combined in one reading and the procedure was repeated five times. They found the least variation with samples of 19 L. However, they chose 8 L samples for their studies, since this volume gave a workable yield of nematodes per litre to permit relatively accurate population estimates.

Faulkner & Bolander (1966) collected samples with a depth-adjustable sampling tube. The tube was attached by a hose to a neoprene impeller pump. The sample size was controlled by pumping timed volumes through stacked 500 µm and 25 µm sieves. The residue on the 25 µm sieve was washed into a container and taken to the laboratory for further analysis. During a survey of the Columbia basin in eastern Washington state, USA, a continuous flow chemical centrifuge was used for taking water samples. It was fitted with a manganese bronze 127 mm solid basket head to concentrate three separate samples of 18,9 L (5 US gallons) each to 300 ml from which particulate matter was flocculated (Faulkner & Bolander, 1970a).

During systematic sampling for plant pathogens in irrigation water in the North Platte Project of Nebraska, nematodes were also recorded. Nematodes were collected in a small volume of water with a 125 µm plankton tow net (Steadman *et al*., 1975). During a survey of irrigation canals in the Gangetic plains in India, 3 x 30 L samples were taken by dipping a 1 L jar about one metre from the bank of the canal (Waliullah, 1984). Each 1 L jar was poured through a 25 µm sieve at each location to concentrate the nematodes. Waliullah (1989) took three 50 L samples at hourly intervals at each sampling site in the Kashmir Valley in India. The nematodes were again concentrated by pouring the water through 25 µm sieves. Roccuzzo & Ciancio (1991) took 10 samples in 10 L containers in an irrigation canal in Italy. The nematodes were concentrated by passing each sample through a 710 µm and a 50 µm sieve and the nematodes collected by backwashing the sieves.

Cohn (1976a) used an apparatus which consisted of a 75 mm diameter suction hose with foot valve, which was pushed into the river or canal to a specific depth. A centrifugal pump supplied water to two smaller hoses. One hose was used to regulate the flow of water onto the bank of metal sieves served by the second hose. Hoses were fitted with a flow meter to quantify sample size. A bank of three sieves stacked in order of 210 µm, 25 µm and 25 µm apertures were used to concentrate each sample. To avoid clogging of the sieves when the water was very dirty, an additional 150 µm sieve was inserted between the 210 µm and the first 25 µm sieve. Smith & Van Mieghem (1983a) used 10 x 20 L plastic containers for sampling by dipping the containers in the water. The containers were left for two days in the laboratory to settle and the supernatant siphoned off. This gave adequate concentration without loss of nematodes.

B. Where to take water samples

To determine the distribution of nematodes in an irrigation canal in Washington state, USA, samples were taken at different depths, and distances from the bank, and the water velocities at these points were measured

(Faulkner & Bolander, 1966). They found that the numbers of nematodes passing a specific point varied directly with the water velocity, but the density of the nematodes within the canal was random with little tendency to settle out in flowing water.

Faulkner & Bolander (1970a) found no PPN in canals receiving no runoff water, but high numbers of nematodes lower down the basin, where runoff from fields enters the canal. They also found that water containing PPN enters the Potholes Reservoir, but no PPN were detected in the water leaving the reservoir. This suggests that reservoirs and dams could serve as a trap for nematodes. Waliullah (1989) took samples from an upland and lowland canal in the Kashmir Valley in India. He found higher numbers of PPN in the lowland canal (where agriculture takes place) than in the upland canal (upstream from agricultural activities).

Six surface irrigation ponds in southern Georgia were surveyed for plant pathogens by taking samples from the top, middle and bottom of the pond (Shokes & McCarter, 1979). Low populations of PPN were found only in the bottom sediment of the ponds.

C. When to take water samples

Since rivers and irrigation canals are mainly infected with PPN from the runoff water from neighbouring production fields, it can be expected that contamination of irrigation water will show a seasonal effect. This must be taken into consideration in timing surveys for the presence of PPN in irrigation water. This is supported by the results of Faulkner & Bolander (1966; 1970a), Cohn, (1976a) and Waliullah (1984), who indeed found high nematode numbers in irrigation water during rainy periods, when runoff was high.

Producers need reliable guidelines for sampling of irrigation water used in the production setup to be able to detect and monitor for the presence of PPN. The problem with all detection techniques is the interpretation of negative results. Especially in the case of water samples, the question arises if the nematodes were completely absent or were they present in such low numbers that the sampling method failed to detect them. The above-mentioned studies also show the importance of sampling irrigation systems at the correct point, otherwise PPN numbers will be grossly underestimated.

5. SURVIVAL AND INFECTION POTENTIAL OF PLANT PARASITIC NEMATODES FROM WATER SOURCES

Faulkner & Bolander (1970b) used methyl bromide fumigated soil planted with a series of suitable hosts and irrigated it with either well water or contaminated canal water. They demonstrated that PPN introduced to non-infected land via contaminated canal water can establish very successfully and are an important source of nematode infestation, with devastating results after the second season.

It has been assumed that PPN may not be able to survive long periods of submersion in water in the absence of a food supply and without the rapid exchange of gasses (Wallace, 1971). However, contrary reports appeared in the literature (Table 2), and also from research performed in open and closed soilless culture systems. Waliullah (1984) showed that nematodes could survive at least 15 days. *Tylenchorhynchus*, *Pratylenchus* and *Helicotylenchus* collected from canals at Charar-e-Sharief, Badgam survived in a sample of water for 70 days (Waliullah, 1989).

Second stage larvae of *M. javanica* stored in water at 15ºC for16 days retained a high percentage of motility and infectivity (Thomason *et al*., 1964). Moens & Hendrickx (1993) kept *M. incognita* larvae in water at 2, 18 and 25ºC. They found that the ability of *M. incognita* to survive a prolonged stay in water is temperature dependant: 2ºC being lethal after 7 days; at 18ºC and 25ºC the decrease of reproductivity can be associated with the decrease of both motility and lipid reserves. Reproductivity decreases after two weeks.

Smith & Van Mieghem (1983a) investigated the survival of handpicked *Xiphinema* spp. in dam and tap water by submerging the nematodes in cages constructed of 50 mm ring sections of 50 mm diameter PVC piping with the open ends covered by gauze of 10 µm pore size to prevent the nematodes escaping. Survival of *X. index* seemed better in dam and river water than in tap water under laboratory conditions. The longest period of survival under natural conditions was 13 days.

Table 2 Tests for survival and infectivity of PPN in water (*nematodes attacking vines in bold*)

Nematode	Origin of sample	Water source	Days of survival	Tested for infectivity	Reference
Meloidogyne javanica	Glasshouse culture	Laboratory	**16-32**	Yes Yes	Thomason *et al*., 1964; Van Gundy *et al*., 1967
Meloidogyne incognita	Glasshouse culture	Laboratory	**14**	Yes	Moens & Hendrickx, 1993
Plant parasitic nematodes	Upper Gangetic Canal, India	Irrigation canal	15	Yes	Waliullah, 1984
Plant parasitic nematodes	Spain	Irrigation canal	64	Yes	Tobar-Jimenez & Palacios-Mejia, 1976
Rhadopholus similis	Glasshouse cultures	Laboratory Hydroponic system	52 -	No Yes	Birchfield, 1957 Van Os *et al*., 1999
Tylenchorhynchus, ***Pratylenchus***	Charar-e-Sharief, Badgam, India	Irrigation canal	**70**	No	Waliullah, 1989
Tylenchulus semipenetrans	Glasshouse culture	Laboratory	**128**	Yes	Van Gundy *et al*., 1967
Xiphinema index	Glasshouse culture	Dam water	**13**	No	Smith & Van Mieghem, 1983a

6. MANAGEMENT OF PLANT PARASITIC NEMATODES IN IRRIGATION WATER

Literature shows that standard water treatment procedures that are successful for the elimination of various other pathogens do not always work for nematodes. Nematodes have an inherent capacity to survive antagonistic environments, which explains the successful adaptation of the phylum Nematoda to the diverse variety of habitats it occupies.

With an increasing global human population, South Africa is no exception in being forced into more intensified use of agricultural land. Higher production of food in itself will lead to an increase in contamination by nematodes of runoff and surface water used for irrigation.

Water is not the natural habitat of PPN and irrigation water becomes contaminated only incidentally along the way. Before any water treatment method is considered, all possible steps should be taken to prevent the initial water source from becoming contaminated with PPN. Only when a clean water source is not available or nematodes cannot be prevented from entering the irrigation system, should physical, chemical, or combinations of treatments be considered (Table 3).

Table 3 Possible treatments for the control of PPN in irrigation water

Type of control	Reference
Chlorination	Grech & Rijkenberg, 1992; Runia 1995
Electrical discharge	Dematte *et al*., 1993
Filtration	Amsing & Runia, 1995; Grech *et al*., 1989; Hallmann *et al*., 2005; Moens & Hendrickx, 1992; Van Os *et al*., 1999;
Heat treatment	Hallmann *et al*., 2005; Runia & Amsing, 2001a, b;
Hydrogen peroxide	Runia & Amsing, 1996
Ozonation	Moens *et al*., 1991; Runia & Amsing, 1996
Sedimentation and flocculation	Amsing & Runia, 1995; Hallmann *et al*., 2005
Ultra violet radiation	Amsing & Runia, 1995; Grech *et al.* 1989; Hallmann *et al*., 2005; Moens & Hendricks, 1989; Pieterse & Van Mieghem, 1987; Runia, 1994
Combination of methods	Grech *et al*., 1989

A. Chlorination

With an improved electrolytic method of chlorine gas generation, the cost of commercial treatment of irrigation water for the elimination of pathogens has now become economically justifiable. Effective chlorination depends on the exposure time of the organism to free chlorine, the quality, chlorine content and pH of the water. High chlorine levels may be phytotoxic to certain crops or can restrict root development (Runia, 1995).

Water from the Crocodile River, Mpumalanga, South Africa, infested with nematodes (30% *Tylenchulus semipenetrans*), pumped into a holding dam, was used to test the effect of chlorination of irrigation water on nematodes (Grech & Rijkenberg, 1992). Free chlorine was maintained at 40-55 $\mu g.ml^{-1}$ for 11 min. It was found that the nematode numbers in the irrigation water passing through the emitter were not affected by the chlorination treatment. It was also shown in the laboratory that *T. semipenetrans* exhibits a high tolerance to free-chlorine levels of up to 50 $\mu g.ml^{-1}$. These results indicated that at least some nematode species cannot be effectively controlled solely by the chlorination of irrigation water alone.

B. Electrical discharge

The only reference to this type of control was by Dematte *et al*. (1993). They tested the effect of electrical discharges without thermic effect, and of energy fields to control *M. incognita* race 1 larvae in weir water. The result showed a 10% kill of larvae attributed to the electrical discharge. Many claims have been made about the use of electrical discharge to control PPN, but no research in this regard has been done in South Africa.

C. Filtration

Water filters of different porosities and designs are currently available. The main limitation of filters is the high maintenance required because of clogging by the organic matter present in irrigation water. To eliminate nematodes, especially the small larvae of root-knot and citrus nematodes, filters with a pore size of 5 µm are essential. This makes it very difficult to get the required amount of water necessary for farming operations through the system in the available time. Usually a series of filters are needed, starting with a sand filter to eliminate the bulk of organic matter, followed by filters in decreasing pore size. Many types of filters can be used with progressively smaller pore size after a sand filter. These filters are made from synthetic materials formed into flat sheets, pleated sheets or cylinders. Most of these units need pre-filtering or sedimentation in a storage dam or reservoir to prevent clogging and maintain good flow rates.

Slow sand filtration has been used for over a century as part of the purification process of drinking water treatments (Ellis, 1985). It is, however, not completely effective for the removal of PPN from irrigation water because of the large pore size. Van Os *et al*. (1999) used slow sand filtration with three types of sand: fine (0,15-0,35 mm), medium (0,20-0,80 mm), and coarse (0,50-1,60 mm), in a closed hydroponic system. *Radopholus similis* was added to the water and elimination was checked by sampling the filtrate over a period of 21 days. Low concentrations of *R. similis* were detected in the water for up to 21 days of recirculation through the filter system. Sand filtration can, however, serve as the first step in a combination filter system to remove most organic contamination products from the water. This would give better quality water for further filtration or high-tech treatment. Slow sand filters are not expensive and can be easily built and maintained (Hong & Moorman, 2005).

To eliminate nematodes from the drainage water of a hydroponics-type system, Moens & Hendrickx (1992) built a customised filter unit. The unit included two sedimentation reservoirs, from which the water passed through a series of four filters comprising a gauze cartridge (150 µm) and three polyester felt filter bags (one of 80 µm and two of 1 µm each). The second stage juveniles of *Globodera rostochiensis* were used as test organisms. By using the customized filter, they were able to retain all *G. rostochiensis* juveniles within their filter system.

In a closed hydroponic system, reverse osmosis (hyper filtration) is not suitable because the fertilizers in the water have to be re-used. Membranes used in ultra filtration, however, let fertilisers through, and will effectively remove nematodes from irrigation water because of the pore size. Membranes used in reverse osmosis and ultra filtration are not recommended for disinfestations of irrigation water, because of clogging (Runia, 1995).

Grech *et al*. (1989) designed a system to eliminate pathogens from citrus nursery irrigation water. This consisted of a pump, a swimming pool sand filter, a series of three cartridge filters with porosities of 100 µm, 20 µm and 5 µm and an ultraviolet sterilisation unit. The unit had a maximum water delivery of 1 500 L/h. The system was designed to eliminate fungi and bacteria from the irrigation water, but citrus nematodes in the river water were also eliminated following filtration and irradiation. The nematodes were removed during filtration, as the UV radiation level used in the system was too low to affect the nematodes (More, 1973).

At the Plant Quarantine Station in Stellenbosch it was found that plants planted in steam sterilized soil and kept under quarantine conditions became infected with ring, lesion and root-knot nematodes. This showed that the municipal water used for irrigation of the plants was infested with these nematodes (Smith & Van Mieghem, 1983a). The installation of filters with a nominal aperture size of 5 µm to eliminate nematodes from municipal water before irrigating glasshouse propagation material solved the problem effectively.

D. Heat treatment

Heat treatment of plant material such as roots, bulbs and whole plants has successfully been used for the elimination of endoparasitic nematodes such as root-knot, lesion and burrowing nematodes. Generally, nematodes in plant material cannot survive temperatures above 45^{o}C for longer than 30 min. Direct heat treatment of water is also very effective to kill nematodes in irrigation water (Hallmann *et al,*. 2005).

In Europe the recommendation to eliminate pathogens from circulated water in closed soilless culture systems was to treat the water for at least 30 s at 95^{o}C (Runia & Amsing, 2001a). However, all the beneficial organisms are also killed. Runia & Amsing (2001b) used a laboratory setup to determine the optimum

temperature to kill *R. similis* (burrowing nematode). A temperature of 48°C for 5 min or 50°C for 2 min or 53°C for 30 s, respectively, was found to be lethal for *R. similis*. For simultaneous control of plant pathogenic fungi, bacteria and nematodes, they recommended a temperature of 60°C for 2 min for recirculation water.

E. Hydrogen peroxide treatment

Only one reference in the literature on the use of hydrogen peroxide for the control of nematodes in irrigation water was found. Runia & Amsing (1996) tested activated hydrogen peroxide for the control of *R. similis*. They found that hydrogen peroxide was effective at a concentration of 400 ppm or higher for at least 24 hours. With a different formulation, only 200 ppm for 24 hours was required to completely eliminate *R. similis* (Runia & Amsing, 1996).

F. Ozonation

The commercial source of ozone is the 'ozonator'. Ozone (O_3) is a three atom allotrope of oxygen and is an extremely effective oxidant. Ozone will oxidise both organic and inorganic substances and the ozone decomposes in a matter of hours to 'normal' oxygen (O_2).

Moens *et al*. (1991) examined the sensitivity of *M. incognita* second stage juveniles to ozone treatment in the laboratory. They found that oxidation for 4 min totally inhibited the nematode infection potential on tomato plants, but complete nematode kill was only achieved after a treatment time of 12 min. The level of redox potential (750 mV) was not an effective measurement for disinfestation ability in drain water due to an abundance of other oxidisable compounds.

Ozone was also tested for its effectiveness against the burrowing nematode, *R. similis*. Runia & Amsing (1996) found that an exposure time of at least one hour to ozone was required to eliminate *R. similis* and prevent its reproduction on *Anthurium andreanum* in the laboratory. Exposure time was used to measure the infective ability of ozone.

A disadvantage of ozone is that water has to be batch treated in holding tanks, because of the time it takes to achieve the correct redox value. The pH of the water must be acidic (pH 4) to provide greater ozone stability. The efficacy of ozone also depends on the oxygen demand of the drainage water, and the nature of the oxidation compounds (Runia, 1995; Runia & Amsing, 1996).

G. Sedimentation and flocculation

If water with suspended nematodes is allowed to stand, the nematodes will settle to the bottom. This settling process may be accomplished by pumping water into a settling dam or reservoir and leaving it for some time

for the nematodes to settle to the bottom. The outlet should be mounted on a floating device as far as possible from the inlet.

Moens & Hendrickx (1992) demonstrated the usefulness of filtration techniques for effective nematode control in laboratory and practical experiments. A filter unit was built in which the drain water was caught in a sedimentation reservoir from which it was pumped (1 000 L/h) into a second tank, passing through a series of four filters comprising a gauze cartridge (150 µm) and three polyester felt filter bags (80, 1 and 1 µm). All PPN were retained by this filter system.

H. Ultraviolet light

Ultraviolet (UV) light falls between visible light and x-rays in the spectrum of electromagnetic radiation. As such, in the short wave band of 200 – 280 nm (UVC rays), its radiation generates a strong germicidal effect.

The sensitivity of *M. incognita* juveniles to UV-rays was examined under laboratory conditions by Moens & Hendrickx (1989). They found that 50% of the juveniles were immobilized at an irradiation dose of 142 $mJ.cm^{-2}$. They also found that a dose of 14 $mJ.cm^{-2}$ had no apparent effect on the nematodes, but it completely inhibited the nematodes from infecting tomatoes. Direct killing of *M. incognita* second stage juveniles was only achieved at dosages as high as ≥200 $mJ.cm^{-2}$. Amsing & Runia (1995) reported similar results for *R. similis,* where a dose of 10 $mJ.cm^{-2}$ completely inhibited reproduction. The dosage depends on the water flow rate and UV doses of 100 $mJ.cm^{-2}$ are recommended for the control of PPN when drain water passes the UV unit at 2,5 m^3/h flow rate (Moens & Hendrickx, 1989). However, in hydroponic-like systems some minerals like manganese and magnesium in the nutrient solution become unstable because of the UV light and precipitates, causing nutrient deficiencies (Moens & Hendricks, 1989).

Second stage juveniles of *M. javanica* were used to test a commercial UV apparatus (Pieterse & Van Mieghem, 1987). The root-knot juveniles were exposed to different levels of UV, and were then used to inoculate tomato seedlings in steam sterilized potting soil. After exposure times of 3 to 5 min to UV, no reproduction was observed on the inoculated tomato seedlings after a period of two months.

UV as a possible method to kill nematodes in irrigation water has no effect on the environment and would be preferable to chemical treatment. The main limiting factor in the use of UV is the quality of the water to be treated. This can be achieved by pre-filtering the water (Grech *et al*., 1989; Runia, 1994).

I. Combination of methods

A combination of UV irradiation and filtration of irrigation water was investigated for the control of pathogens in irrigation water of citrus nurseries (Grech *et al*., 1989). The UV unit delivered a minimum

energy level of 30 000 $mWs.cm^{-2}$ at a wavelength of 254 nm on the lower surface of the exposure chamber. According to More (1973) the UV radiation level used in the system would have been too low to affect the nematodes. The raw water tested with this system contained citrus, lesion and root-knot nematodes. No nematodes were observed following the filtration and irradiation. This can be attributed to the cartridge filters used, which effectively eliminated the nematodes (Grech *et al*., 1989)

7. CONCLUDING REMARKS

The citrus industry in South Africa made major strides since 1976 in preventing plant pathogens contaminating irrigation water. Research was undertaken, concentrating on the control of pathogens, including nematodes, mainly the citrus nematode, in irrigation water used in citrus nurseries (Hough, 1979; Grech & Frean, 1987; Willers & de Jager, 1987; Grech *et al*., 1989; Grech & Rijkenberg, 1992).

Relatively few surveys had as their main aim the detection of PPN emanating directly from irrigation water (Faulkner & Bolander, 1966, 1970a, b; Smith & Van Mieghem, 1983a; Waliullah, 1984; 1989), compared to the detection of other plant pathogens in water (Hong & Moorman, 2005). However, from the results of these few surveys overwhelming evidence was obtained of the danger of introducing PPN to production sites by means of irrigation water. Although the nematode numbers in most cases seemed relatively low with regard to the sample size, in the context of the volume of water irrigated an enormous number of nematodes are repeatedly introduced throughout the growing season, especially after heavy rain.

With the movement from soil to soilless culture systems, PPN were thought to be excluded. As these nematodes were not expected to be found in soilless culture they were easily overlooked, with devastating results to the crops involved. The detection of PPN in soilless culture has sparked much research on the pathways of contamination, and on the control of PPN in drainage water (Hallmann *et al.*, 2005). A closed hydroponic system is also an ideal experimental setup to test the success of different control methods on nematodes.

South Africa is heavily dependent on surface water for irrigation, and as agriculture intensifies the problem of contaminated irrigation water will increase. Each production site should be evaluated with regard to the water source used for irrigation. All possible ways of contamination should be examined and measures taken to prevent it. Only as a last resort, after all measures to prevent contamination have been taken, should other means of control be considered.

From the research done on the treatment of nematodes in irrigation water it has become clear that no single method will suffice to eliminate all PPN from irrigation water. Chlorination, although very effective against bacteria and fungi, does not seem to be a solution for the elimination of nematodes from irrigation water

without phytotoxicity to the host plant. Heat treatment can be viable in a small hydroponic system, but is not cost-effective. Electrical discharge was tested as a possibility, but it is still in the research stage. Filtration with a series of filters of different porosity, in combination with other methods such as ozone or UV radiation, seems to be a viable possibility. Different water treatment options are available (Table 3) and various options are employed successfully in closed hydroponic systems. Each strategy has advantages and disadvantages with regard to maintenance, costs and benefits. To use some of these treatment options singularly or in combination, it is important that specialists (manufacturers, producers, and researchers) should come together to solve this complex problem.

The control of nematodes and other pathogens in irrigation water should be recognized alongside pest and disease control as a significant crop health issue and should form an integrated part of any integrated pest management system.

8. PROPOSALS FOR FUTURE RESEARCH

There is a great need for further research on the subject of PPN in irrigation water in South Africa with regard to the following:

A. Surveys

In South Africa only three surveys for the presence of PPN in irrigation water have been done (Cohn, 1976b; Smith & Van Mieghem, 1983a, b). More surveys are needed to determine the occurrence and fluctuations of PPN in waterways and surface water in South Africa. The research is needed to determine when and where water samples for PPN should be taken and the role irrigation water plays in the re-infection of fumigated soil and new plantings.

B. Sampling methods

Effective and reliable sampling methods for the presence of PPN in water samples need to be developed. Researchers, producers and farmers need simple and reliable sampling methods to detect and monitor for the presence of PPN in a specific irrigation setup. Techniques to detect nematodes in irrigation water are also needed to justify the cost and management of the implementation of control strategies.

C. Preventing contamination

In the South African plant improvement certification schemes, especially for grapevines, each production unit used for the production of GFLV-free plant material should be evaluated for possible pathways of contamination of the irrigation water with PPN. Since most water treatment methods, especially for

nematodes, are expensive to establish, all possible steps should be taken to use a water source that is free from the nematodes and prevent nematodes from entering the water. Pathways for possible contamination of irrigation water have to be investigated and simple solutions found to rectify it. Examples are basic sanitation procedures such as preventing potted plants to come in contact with the floor in a greenhouse setup, cleaning water by using the settling process through the use of primary and secondary dams, preventing contaminated soil or crop debris reaching the water, and the placement of intake and outflow pipes in dams and reservoirs. All these aspects should be examined to determine their contribution towards contaminating irrigation water.

D. On site management plans

In the South African citrus industry the potential for contamination of nursery material with nematodes such as *T. semipenetrans* and plant pathogens such as *Phytophthora* was realized at an early stage (Cohn, 1976b; Grech *et al*., 1989). Successful elimination of *T. semipenetrans* and *Phytophthora* from nurseries was obtained by a combination of different methods, including managerial, physical and chemical methods.

Nursery irrigation water has to be pest- and disease-free to prevent the contamination of plant material. Only when the water source is a borehole can it be assumed that the risk of contamination with nematodes is low. All nurseries in South Africa are obliged to supply plant material of a high phytosanitary standard to the growers. This can only be done if the irrigation water is guaranteed to be free from PPN.

Each farm or production site differs in the following aspects:

- Physical and chemical quality of the water
- Quantity of water needed in a specific time frame
- Cultural practices
- Susceptibility to pests and diseases of the crop produced.
- Economic resources
- Management options

Handling and treatment of nematodes in irrigation water in every production setup is unique and management strategies must be designed to fit each case. As information becomes available for specific cases, new cases should be easier to address.

E. Commercial production units

Various managerial options are available for the control of nematodes in irrigation water in small production units. Most of these options are not suitable for farms and commercial production units where large quantities of irrigation water are needed. Research teams consisting of nematologists, plant pathologists,

farmers, and manufacturers of specific apparatus such as UV radiators, ozonators and filter systems should come together to solve such a complex problem.

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