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Validation of the specificity and sensitivity of species-specific primers that provide a reliable molecular diagnostic for *Xiphinema diversicaudatum*, *X. index* and *X. vuittenezi*

Judith Hübschen[1], Lilo Kling[1], Ulrike Ipach[1], Volker Zinkernagel[2], Nathalie Bosselut[3], Daniel Esmenjaud[3], Derek J.F. Brown[4] and Roy Neilson[5,*]
[1]*Dienstleistungszentrum Ländlicher Raum-Rheinpfalz, Breitenweg 71, 67435 Neustadt/Weinstrasse, Germany;* [2]*Technische Universität München, Lehrstuhl für Phytopathologie, Am Hochanger 2, 85350 Freising Weihenstephan, Germany;* [3]*Institut National de la Recherche Agronomique, BP 2078, 06606, Antibes, France;* [4]*Central Laboratory of General Ecology, 2 Gagarin Street, 1113 Sofia, Bulgaria;* [5]*Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK; *Author for correspondence (Phone: +44-1382-562731; Fax: +44-1382-562426; E-mail: roy.neilson@scri.sari.ac.uk)*

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Abstract

Xiphinema diversicaudatum and *X. index* are vector nematode species of economic importance in viticulture regions as they can transmit Arabis Mosaic, Grapevine Fanleaf and Strawberry Latent Ringspot viruses to grapevine. Wang et al. (2003) designed species-specific diagnostic primers from ribosomal genes for both these vector species as well as a vector and a non-vector species *X. italiae* and *X. vuittenezi*, respectively. Our study aimed to confirm the specificity and determine the sensitivity and reliability of the primers for the two vector species, *X. diversicaudatum* and *X. index* when challenged with closely related longidorid species and general nematode communities typical of vineyard soil. With one exception, no PCR product was observed when the primers were tested against six *Longidorus*, one *Paralongidorus* and one *Xiphinema* non-target species. Occasionally (three out of eight replicate PCR reactions) a weak PCR product was noted when primers for *X. index* were tested with *L. elongatus*. Furthermore, when challenged with a range of non-target nematode species comprising the nematode community typical of viticulture soil, no PCR product was amplified. An experimental dilution series of extracted DNA rigorously demonstrated that DNA from an equivalent single specimen of the target virus-vector species, *X. diversicaudatum* and/or *X. index*, could be detected amongst 1000 equivalent non-target *X. vuittenezi*. Also, extracted DNA from an equivalent single target specimen was detected when added to DNA extracted from the overall soil nematode community. The primers were assessed further by using serial mixtures of actual nematodes rather than extracted DNA to simulate field soil. Using this method, a single target nematode could be detected amongst 200 non-target specimens. Given their specificity, sensitivity and reliability, it appears that these diagnostic primers will be of great benefit to phytosanitary/quarantine services related to the viticulture industry.

Introduction

As with the majority of viticulture regions around the world, many German vineyard soils contain species of economically important longidorid nematodes that are vectors of nepoviruses. In particular, *Xiphinema index* is known to transmit grapevine fanleaf virus (GFLV) and *X. diversicaudatum* transmits both arabis mosaic virus (ArMV) and strawberry latent ringspot virus (SLRSV) (Hewitt et al., 1958; Harrison and Cadman, 1959; Jha and Posnette, 1959; Lister,

1964). All three viruses have been found in grapevines (Hewitt, 1950; Vuittenez et al., 1970; Rüdel, 1978). However, the most common *Xiphinema* species in vineyard soils from Germany is *X. vuittenezi* that has been suspected to transmit both ArMV and GFLV (Flegg, 1969; Rüdel, 1980), but has not yet been unequivocally proven as a virus-vector following the criteria published by Trudgill et al. (1983).

According to German and most other European viticulture regulations, grapevines used for multiplication must be grown in soil free from virus-transmitting nematodes. It is therefore essential to clearly distinguish between the two recognised *Xiphinema* virus vector species and other *Xiphinema* spp. that are not virus-vectors, e.g. *X. pachtaicum* and *X. vuittenezi*. Currently species determination is based on morphological characters and morphometric data of mainly adult females (Chen et al., 1997; Loof and Chen, 1999). Frequently, population densities are low, and if only juveniles of more than one species occur in the same soil sample reliable identification is problematical. Furthermore, the number of classical nematode taxonomists, as with taxonomists in other disciplines, is rapidly decreasing (Behan-Pelletier, 1999; Andre et al., 2001). Consequently, as a surrogate to classical taxonomy, molecular diagnostics provide the opportunity to have simple and reliable alternative methodologies for the determination of economically important nematode species (Jones et al., 1997; Powers et al., 1997; De Giorgi et al. 1999).

Compared with other plant-parasitic genera such as *Globodera* (for example, Roosien et al., 1993; Mulholland et al., 1996; Shields et al., 1996; Fleming et al., 1998; Fullaondo et al., 1999; Vejl et al., 2002), *Heterodera* (Subbotin et al., 2000; Zheng et al., 2000; Amiri et al., 2002), *Pratylenchus* (Orui and Mizukubo, 1999; Uehara et al., 1998, 1999; Waeyenberge et al., 2000) and especially *Meloidogyne* (for example, Zijlstra et al., 1995, 1997, 2000; Zijlstra, 1997, 2000; Petersen et al., 1997; Williamson et al., 1997; Orui, 1998; Dong et al., 2001; Wishart et al., 2002 and references therein), molecular diagnostics for *Xiphinema* have been comparatively ignored.

Restriction fragment length polymorphisms (RFLPs) have been used to separate populations of both putative *X. americanum* group and non-*X. americanum* group species (Vrain et al., 1992; Vrain, 1993; Knoetze et al., 2000). Furthermore, Molinari et al. (1997) obtained amplification products from individual specimens of *X. index* and *X. vuittenezi*, using the primers developed by Vrain et al. (1992). The resultant PCR fragments showed differences in size between the two target species (ca. 2000 and 1800 bp for *X. index* and *X. vuittenezi*, respectively) compared to populations belonging to the *X. americanum* group (ca. 1500 bp). However, given that the genus *Xiphinema* comprises >200 species (see Figure 4.1, Taylor and Brown, 1997) there is a definite likelihood that a single universal primer pair would not discriminate between all target and non-target species of the genus.

Recently, Wang et al. (2003) published species-specific primers for *X. diversicaudatum*, *X. index*, *X. italiae* and *X. vuittenezi*. These primers can be used in a multiplex test as they yield different sized PCR fragments utilising the same reverse primer thus being efficient, and inexpensive to use. Crucially, the primers (Wang et al., 2003) were sufficiently sensitive to detect all developmental forms of these nematodes. Whilst Wang et al. (2003) validated the specificity of the primers against the four target *Xiphinema* species, they did not expand the validation process to other longidorid species that frequently occur in vineyard soils. For example, a further fifteen non-target longidorid species are known to occur in German vineyards. Absent from the study of Wang et al. (2003) was the determination of primer sensitivity, for example, when challenged to identify low densities of a target nematode within a larger non-target nematode inoculum, analogous to testing field samples as opposed to artificially laboratory created scenarios (1:1 ratio). Finally, although absolutely necessary for their study, the 'hand-picking' of target nematode species for analysis by Wang et al. (2003) assumes *a priori* basic knowledge of nematode taxonomy that as previously noted is rapidly diminishing. Therefore for routine use, in a general diagnostic laboratory by non-nematode specialists, these species-specific primers should be validated against the complete nematode community typically found in soils of the source habitat.

The objectives of this study were therefore threefold: (a) to investigate whether the species-specific primers designed by Wang et al. (2003) have any cross-reaction with non-target longidorid

species that are known to occur in German vineyards; (b) to assess the sensitivity of the primers targeted for two virus-vector species, *X. diversicaudatum* and *X. index* by performing a series of dilution-type experiments with both extracted DNA and whole nematodes combined with the most prevalent non-vector *Xiphinema* species in German vineyards, *X. vuittenezi* and (c) to determine whether DNA and/or whole nematode specimens of the two target virus-vector nematode species could be identified amongst bulk DNA extracted from a typical total nematode community from vineyard soil.

Materials and methods

Non-target nematode specificity

To test for cross-reactions with non-target nematode species, each of three species-specific primers (Wang et al., 2003) for *X. diversicaudatum*, *X. index* and *X. vuitenezi* (*X. italiae* is not present in German vineyards) were tested with various populations of *Longidorus attenuatus*, *L. elongatus*, *L. helveticus*, *L. macrosoma*, *L. profundorum*, *L. sturhani*, *Paralongidorus maximus* and *X. pachtaicum* found in German vineyards (Table 1). Also, two distinct nematode communities that contained a range of trophic groups as defined by Yeates et al. (1993) including saprophagus, predatory and other plant-parasitic nematode species that typically occur in vineyard soil were sampled (Table 1). For each PCR reaction, an individual specimen of the non-target longidorid species was randomly selected from each of the test populations and challenged with the three species-specific primers, thus providing replication across PCR reactions. The number of replicates being equivalent to the number of populations listed in Table 1, with the exception of *L. elongatus* where eight separate PCR reactions in total were performed when a non-specific band was observed from a specimen from the Bad Dürkheim population. Nematodes were either used directly after extraction from soil or alternatively stored in 1 M NaCl at −20 °C until required. As specimens are often heat-killed for morphological examination, we investigated whether this treatment had an influence on DNA extraction and subsequent PCR amplification methods.

Table 1. Longidorid populations used to determine specificity of species-specific primers designed by Wang et al. (2003)

Nematode species	Population	Origin (winegrowing area)
L. attenuatus	Gau Algesheim	Rheinhessen
L. attenuatus	Mettenheim	Rheinhessen
L. elongatus	Bad Dürkheim	Pfalz
L. elongatus[1]	Heppenheim	Hessische Bergstrasse
L. helveticus	Horrweiler	Nahe
L. helveticus	Stadecken	Rheinhessen
L. macrosoma[2]	Freiburg	Baden
L. macrosoma	Godramstein	Pfalz
L. macrosoma[1]	Heppenheim	Hessische Bergstrasse
L. macrosoma	Knöringen	Pfalz
L. profundorum	Gau Algesheim	Rheinhessen
L. profundorum	Horrweiler	Nahe
L. profundorum	Landau	Pfalz
L. sturhani	Gau Algesheim	Rheinhessen
L. sturhani	Ockenheim	Rheinhessen
P. maximus	Bad Dürkheim	Pfalz
P. maximus	Deidesheim	Pfalz
P. maximus	Hambach	Pfalz
P. maximus	Mußbach	Pfalz
X. diversicaudatum	Hambach	Pfalz
X. diversicaudatum	Ilbesheim	Pfalz
X. diversicaudatum	Piesport	Mosel
X. diversicaudatum	Schweigen-Rechtenbach	Pfalz
X. index	Bad Dürkheim	Pfalz
X. index	Bissersheim	Pfalz
X. index	Freiburg	Baden
X. index	Haardt	Pfalz
X. index[3]	Iphofen	Franken
X. index	Klotten	Mosel
X. index	Nierstein	Rheinhessen
X. pachtaicum	Gau Algesheim	Rheinhessen
X. pachtaicum	Landau	Pfalz
X. vuittenezi	Bad Dürkheim	Pfalz
X. vuittenezi	Bad Kreuznach	Nahe
X. vuittenezi	Bissersheim	Pfalz
X. vuittenezi	Deidesheim	Pfalz
X. vuittenezi	Freinsheim	Pfalz
X. vuittenezi	Haardt	Pfalz
X. vuittenezi[1]	Heppenheim	Hessische Bergstrasse
X. vuittenezi	Kleinniedesheim	Pfalz
X. vuittenezi	Mörzheim	Pfalz
X. vuittenezi	Obrigheim	Pfalz
X. vuittenezi	Ockenheim	Rheinhessen
X. vuittenezi	Undenheim	Rheinhessen
X. vuittenezi	Westhofen	Rheinhessen
Nematode community 1	Bad Dürkheim	Pfalz

Table 1. (Continued)

Nematode species	Population	Origin (winegrowing area)
Nematode community 2	Deidesheim	Pfalz
Nematode community 3	Herxheim a	Pfalz
Nematode community 4	Herxheim b	Pfalz
Nematode community 5	Landau	Pfalz

[1]Nematodes provided by S. Schütz (Pflanzenschutzdienst Hessen).
[2]Soil samples provided by G. Bleyer (Weinbauinstitut Freiburg).
[3]Soil samples provided by J.V. Herrmann (Bayerische Landesanstalt für Weinbau und Gartenbau).

DNA mixtures for sensitivity tests

Previously extracted DNA from the three tested *Xiphinema* species (two vector, one non-vector species) was mixed in different serial concentrations (Table 2) to investigate the sensitivity of the species-specific primers. In a separate experiment, a more realistic field scenario was simulated by testing the primers on DNA extracted from mixtures of whole nematodes containing varying ratios of the three target species (Table 3). Following Wang et al. (2003), primers were multiplexed and both experiments were replicated at least twice to confirm consistency of the data.

DNA extraction

DNA was extracted from individual nematodes by a modification of the method described by Stanton et al. (1998). A single nematode was placed in 20 µl of 0.25 M NaOH, incubated overnight at room temperature, thereafter heated to 99 °C for 3 min. Afterwards 10 µl of 0.25 M HCl, and 5 µl each of 0.5 M Tris–HCl (pH 8) and 2% Triton X-100 were added and the mixture was incubated for another 3 min at 99 °C. The DNA was either used directly after extraction or stored at −20 °C. The amount of chemicals used to extract DNA was doubled for samples containing many nematodes, e.g., samples 4, 7 and 8 (Table 3).

PCR and gel electrophoresis

Each PCR reaction comprised of a single Ready-To-Go™ PCR Bead (Amersham Pharmacia Biotech), 0.5 µl of template nematode DNA, 1 µl of each primer and sterile, distilled water to a total volume of 25 µl. Amplification was done using a

Table 2. Composition of mixed DNA serial dilutions previously extracted from target and non-target nematodes used to determine the sensitivity of the species-specific primers designed by Wang et al. (2003)

Sample	DNA mixture	Dilution series
1	0.5 µl XI-DNA (or XD-DNA) + 0.5 µl XV-DNA	1:1
2	0.5 µl XI-DNA (or XD-DNA) + 1.0 µl XV-DNA	1:2
3	0.5 µl XI-DNA (or XD-DNA) + 2.5 µl XV-DNA	1:5
4	0.5 µl XI-DNA (or XD-DNA) + 5.0 µl XV-DNA	1:10
5	0.5 µl XI-DNA (or XD-DNA) + 12.5 µl XV-DNA	1:25
6	0.5 µl XI-DNA (or XD-DNA) + 25.0 µl XV-DNA	1:50
7	0.5 µl XI-DNA (or XD-DNA) + 37.5 µl XV-DNA	1:75
8	0.5 µl XI-DNA (or XD-DNA) + 50.0 µl XV-DNA	1:100
9	0.5 µl XI-DNA + 0.5 µl XD-DNA + 0.5 µl XV-DNA	1:1:1
10	0.5 µl XI-DNA + 0.5 µl XD-DNA + 25.0 µl XV-DNA	1:1:50
11	0.5 µl XI-DNA + 0.5 µl XD-DNA + 50.0 µl XV-DNA	1:1:100
12	0.5 µl XI-DNA + 0.5 µl XD-DNA + 75.0 µl XV-DNA	1:1:150
13	0.5 µl XI-DNA + 0.5 µl XD-DNA + 100.0 µl XV-DNA	1:1:200
14	0.5 µl XI-DNA + 0.5 µl XD-DNA + 200.0 µl XV-DNA	1:1:400
15	0.5 µl XI-DNA + 0.5 µl XD-DNA + 300.0 µl XV-DNA	1:1:600
16	0.5 µl XI-DNA + 0.5 µl XD-DNA + 350.0 µl XV-DNA	1:1:700
17	0.5 µl XI-DNA + 0.5 µl XD-DNA + 400.0 µl XV-DNA	1:1:800
18	0.5 µl XI-DNA + 0.5 µl XD-DNA + 500.0 µl XV-DNA	1:1:1000

XD = *X. diversicaudatum*; XI = *X. index*; XV = *X. vuittenezi.*

Table 3. Composition of serial dilutions of DNA extracted from a mixture of target and non-target nematodes used to determine the sensitivity of the species-specific primers designed by Wang et al. (2003)

Sample	Numbers of nematodes used	Dilution series
1	1 *X. index* (or *X. diversicaudatum*) + 1 *X. vuittenezi*	1:1
2	1 *X. index* (or *X. diversicaudatum*) + 10 *X. vuittenezi*	1:10
3	1 *X. index* (or *X. diversicaudatum*) + 25 *X. vuittenezi*	1:25
4	1 *X. index* (or *X. diversicaudatum*) + 100 *X. vuittenezi*	1:100
5	1 *X. index* + 1 *X. diversicaudatum* + 1 *X. vuittenezi*	1:1:1
6	1 *X. index* + 1 *X. diversicaudatum* + 50 *X. vuittenezi*	1:1:50
7	1 *X. index* + 1 *X. diversicaudatum* + 150 *X. vuittenezi*	1:1:150
8	1 *X. index* + 1 *X. diversicaudatum* + 200 *X. vuittenezi*	1:1:200

GeneAmp® PCR System 2700 thermocycler (Applied Biosystems) with the following conditions: 94 °C for 2 min 45 s followed by 40 cycles at 94 °C for 1 min, 57 °C for 45 s, and 72 °C for 2 min, followed by a 10 min cycle at 72 °C and ending with a storage temperature of 4 °C.

PCR products were separated on a 1% agarose gel stained with ethidium bromide in 1×TBE buffer and visualized under UV light.

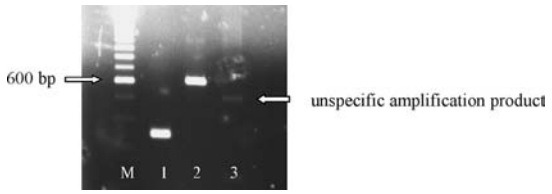

Figure 1. Electrophoresis of the amplification products of DNA extracted from *X. index* (lane 1), *X. vuittenezi* (lane 2) and *L. elongatus* (lane 3). For reaction 1 and 3 the specific primers for *X. index* were used, for sample 2 the specific primer for *X. vuittenezi* (Wang et al., 2003). M corresponds to a 100 bp DNA ladder (GIBCO BRL, Life Technologies).

Results

Reliability of detection

The primers yielded PCR products of the expected fragment sizes (Wang et al., 2003) for all the populations of the three target *Xiphinema* species tested. No differences were observed in subsequent DNA extraction and PCR amplification when using nematodes immediately after extraction from soil, specimens stored in 1 M NaCl or heat-fixed specimens.

Specificity

Primers designed specifically for *X. diversicaudatum* and *X. vuittenezi* when tested with other longidorid species typically found in German vineyards yielded no unspecific amplification products (data not shown). However, the primer pair for *X. index* occasionally (three out of eight replicate PCR reactions) yielded a small amount of an unspecific PCR product resulting in a weak band of approximately 500 bp in length when challenged with *L. elongatus* DNA (Figure 1). No unspecified PCR product was observed when used with DNA extracted from the total nematode communities from Deidesheim and Landau (Table 1).

Sensitivity

The sensitivity of the primers was excellent, as it was possible to clearly detect both *X. diversicaudatum* and *X. index* DNA in a mixture also containing non-vector *X. vuittenezi* DNA across a dilution series ranging from 1:1:1 (or 1:1 when using only two species) to 1:1:800 (Figure 2). At the maximum dilution (1:1:1000) used in this study, only a very weak band for *X. index* could be observed on the gel and the band produced with *X. diversicaudatum* (Figure 2) was almost indiscernible.

In a complementary study, using DNA extracted from whole nematodes of three *Xiphinema* species (two vector, one non-vector) to simulate a more realistic field scenario, the sensitivity of the primers was not diminished. Both target vector *Xiphinema* species could be detected across all tested dilution series (Figure 3) and were comparable to the dilution tests using mixtures of previously extracted DNA.

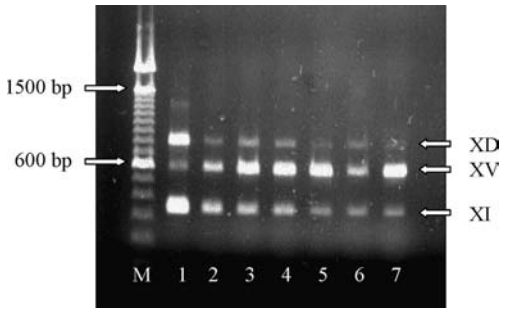

Figure 2. Electrophoresis of the amplification products of DNA mixtures 9, 11, 13, 14, 15, 17 and 18 (Table 2) (lanes 1–7). For all reactions the multiplex species-specific primers described by Wang et al. (2003) for *X. diversicaudatum*, *X. index* and *X. vuittenezi* were used. M corresponds to a 100 bp DNA ladder (GIBCO BRL, Life Technologies); XD: *X. diversicaudatum*; XI: *X. index*; XV: *X. vuittenezi*.

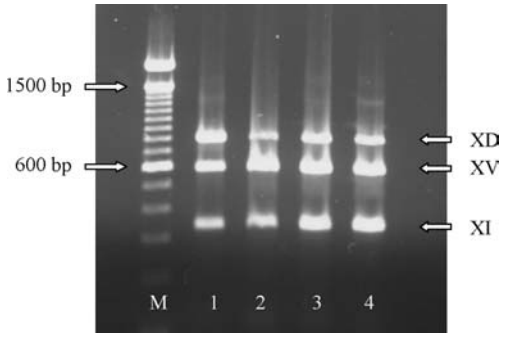

Figure 3. Electrophoresis of the amplification products of DNA extracted from a mix of nematodes (samples 5–8, Table 3) (lanes 1–4). For all reactions the multiplex species-specific primers described by Wang et al. (2003) for *X. diversicaudatum*, *X. index* and *X. vuittenezi* were used. M corresponds to a 100 bp DNA ladder (GIBCO BRL, Life Technologies); XD: *X. diversicaudatum*; XI: *X. index*; XV: *X. vuittenezi*.

Even when a common DNA extraction was carried out with the nematode communities from Herxheim a and b and one specimen each of *X. index* and *X. diversicaudatum*, the specific bands were clearly visible as well as when mixtures made of previously extracted DNA of total nematode communities and the two *Xiphinema* species were used (Figure 4).

Discussion

A molecular diagnostic procedure, designed to discriminate target organisms, that can be utilised

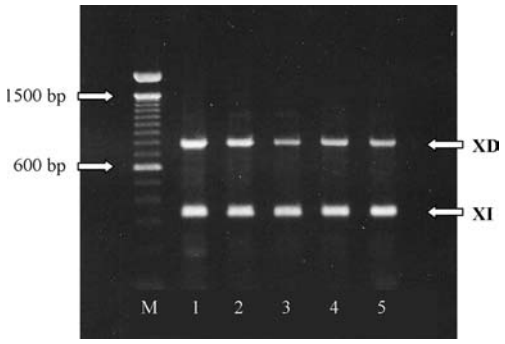

Figure 4. Electrophoresis of the amplification products of DNA: (a) extracted from the nematode communities as listed in Table 1 together with the addition of one each of *X. index* and *X. diversicaudatum* (lanes 1 and 2); (b) previously extracted from nematode communities as listed in Table 1 plus DNA of the two vector species (lanes 3–5) and mixed. For all reactions the multiplex species-specific primers for *X. diversicaudatum* and *X. index* were used (Wang et al., 2003). Lane 1: Nematode community Herxheim a + 1 XI + 1 XD; Lane 2: Nematode community Herxheim b + 1 XI + 1 XD; Lane 3: DNA extracted from Nematode community Deidesheim + DNA extracted from a single XI and XD; Lane 4 and 5: two non-longidorid nematode DNA mixes Nematode community Bad Dürkheim + DNA extracted from a single XI and XD; M = 100 bp DNA ladder (GIBCO BRL, Life Technologies); XD = *X. diversicaudatum*; XI = *X. index*.

in phytosanitary/quarantine laboratories should fulfil three criteria. Firstly, the diagnostic should be reliable and validated against all possible cross-reactions with similar taxa in the habitat under investigation; secondly, it should be a simple test comprised of a single PCR step thus allowing a high throughput of samples and thirdly, be sufficiently sensitive to detect very low numbers of the target organism. Very few of the published nematode diagnostics meet these criteria in full. Furthermore, phytosanitary/quarantine laboratories require a high sample throughput by decreasing time-consuming manipulative procedures to isolate target nematode species, e.g. eliminate hand-picking of putative target nematodes from a soil extract. Thus, the most useful diagnostics are those that have either been demonstrated to detect a single target nematode species within a total soil community comprised of non-target species, as well as producing no cross-reaction products derived from non-target species, or are used in conjunction with a magnetic capture system (Chen et al., 2001, 2003) specific for the target nematode species.

A considerable number of existing diagnostics do not fulfil these criteria as they utilise a two-stage molecular protocol, typically RFLPs (for example, Fleming et al., 1998; Orui and Mizukubo, 1999; Knoetze et al., 2000; Subbotin et al., 2000; Waeyenberge et al., 2000; Zheng et al., 2000; Nguyen et al., 2001) or use duplex PCR (Subbotin et al., 2001). With the continual decrease of classical taxonomic skills (Behan-Pelletier, 1999; Andre et al., 2001), these two-step diagnostic tests may be useful in a research laboratory as a confirmatory test for the presence of a target nematode species. However, they are impractical for use in a phytosanitary/quarantine environment as the methodologies are time-consuming relative to single-step PCR tests, thus consequently more expensive in terms of both human and financial resources. In the future, quantification PCR assays will be utilised but there are currently few such protocols for nematodes (Bates et al., 2002; Holeva et al., 2002).

Currently, several single-step diagnostics are available, although their immediate utility is compromised by a failure to assess their sensitivity, i.e. a dilution series analogous to detecting a single nematode within a range of nematode densities (Petersen and Vrain, 1996; Williamson et al., 1997; Uehara et al., 1998; Blok et al., 2002), or determine their complete specificity, i.e. assessing for cross-reactions with non-target species (Mulholland et al., 1996; Zijlstra, 1997) or both (Shields et al., 1996). A few studies have made a cursory assessment of sensitivity by studying different life stages in conjunction with a test for specificity (Petersen et al., 1997; Zijlstra et al., 2000). Overall, there is a paucity of diagnostics that fulfil the criteria noted above (Fullaondo et al., 1999; Dong et al., 2001; Wishart et al., 2002) although even in those studies, the assessment of specificity was performed on closely related species of the target species but not the entire nematode community typical of the habitat in which the target species would be found.

Wang et al. (2003) developed species-specific primers to detect four *Xiphinema* species typical of vineyard soil and provided clear evidence of no cross-reaction, however, no assessment of sensitivity or complete specificity was made. In Germany, a number of other longidorid species, including *X. pachtaicum* commonly occur in vineyards thus there was a necessity to determine the specificity of the diagnostic test.

Our data confirm that the species-specific primers (Wang et al., 2003) can reliably identify individual specimens of three target *Xiphinema* species (*X. diversicaudatum*, *X. index* and *X. vuittenezi*) originating from the Palatinat region of Germany. Also, that with one exception there were no cross-reactions with any other longidorid species studied. A very weak band was occasionally observed when the specific primers for *X. index* were tested with *L. elongatus* DNA, but the resulting fragment was only approximately 500 bp long. Given that the expected fragment sizes for *X. index*, *X. vuittenezi* and *X. diversicaudatum* are 340, 591 and 813 bp (Wang et al. 2003), there should be minimal confusion. Furthermore, our data have demonstrated that the means of processing the nematodes such as heat killing or storage in 1 M NaCl had no adverse effects on the diagnostic protocol. Additionally, no unspecified PCR products were produced when the primers were exposed to the total nematode communities extracted from vineyard soil. Thus, non-specialists can readily and reliably use these primers in a general diagnostic laboratory.

Longidorid nematodes typically occur in low numbers and frequently as a mixture of vector and predominantly non-vector species (Boag and Topham, 1985; Topham et al., 1985; Oliveira et al., 2003), therefore, it is imperative that species-specific primers are both selective and sensitive. Our data clearly demonstrate that the specific primers for the target virus-vector species *X. diversicaudatum* and *X. index* can detect DNA across a range of concentrations. Using a mixture of extracted DNA, we have demonstrated that it is possible to detect the equivalent of a single target vector species (*X. diversicaudatum* or *X. index*) amongst 1000 target non-vector species (*X. vuittenezi*).

Simulating a nematode population from a vineyard soil by adding varying numbers of vector and non-vector nematode species and thereafter extracting DNA it was also possible to detect a single specimen of a target vector species amongst 200 non-vector specimens. PCR products for both target vector species, *X. index* and *X. diversicaudatum*, at the maximum dilution tested (1:1:200) were intense suggesting that an even higher level of sensitivity could be attained. However, as the number of *X. vuittenezi* found in 100 g of vineyard soil in Germany rarely exceeds 70 or 80 individuals, for practical requirements the method can be

considered sufficiently sensitive (L. Kling, unpublished data).

We have demonstrated that the primers designed by Wang et al. (2003) to detect the virus-vector nematode species *X. diversicaudatum* and *X. index* are species specific, reliable and sensitive when tested with closely related longidorid species and other nematodes that typically occur in German vineyard soil. Furthermore, the diagnostic protocol is a single step PCR and can be considered of benefit to phytosanitary/quarantine services.

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