National Diagnostic Protocol

Wheat Spindle Streak Mosaic Virus

(WSSMV)

NDP 48 V1.0

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Purpose

National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

- are a verified information resource for plant health diagnosticians
- are consistent with ISPM No. 27 – Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs although NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

https://www.ippc.int/core-activities/standards-setting/ispms

Process

NDPs are facilitated and endorsed by the Subcommittee on Plant Health Diagnostics (SPHD). SPHD reports to Plant Health Committee and is Australia's peak technical and policy forum for plant health diagnostics.

NDPs are developed and endorsed according to Reference Standards developed and maintained by SPHD. Current Reference Standards are available at https://www.plantbiosecuritydiagnostics.net.au/work/subcommittee-on-plant-health-diagnostics/

NDPs are living documents. They are updated every 5 years or before this time if required (i.e. when new techniques become available).

Document status

This version of the National Diagnostic Protocol (NDP) for Wheat Spindle Streak Mosaic Virus is current as at the date contained in the version control box below.

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Further information

Inquiries regarding technical matters relating to this protocol should be sent to: sphd@agriculture.gov.au

Contents

1 INTRODUCTION

Wheat spindle streak mosaic virus (WSSMV) is transmitted by the soil-borne plasmodiophoraceous vector, *Polymyxa graminis* Ledingham (Slykhuis and Barr, 1978). While the virus has not been detected in Australia, the vector occurs in Western Australia and Queensland, and as an unconfirmed report, in New South Wales (Cox *et al.,* 2014; Thompson *et al*., 2011).

1.1 Host range

WSSMV infects wheat (*Triticum aestivum*), durum wheat (*T. durum*), triticale (× *Triticosecale*) and rye (*Secale cereale*). WSSMV has not been found to infect barley (*Hordeum vulgare*), nor any dicotyledonous species (Brunt *et al*., 1996 onwards; Jones, 2004, Jezewska and Trzmiel, 2007; Jiang *et al.,* 2020).

2 TAXONOMIC INFORMATION

Realm: *Riboviria*
Phylum: *Pisuviricota*
Kingdom: *Orthornavirae*
Class: *Stelpaviricete*
Order: *Patatavirales*
Family: *Potyviridae*
Genus: *Bymovirus*
Species: *Wheat spindle streak mosaic virus*

Synonyms: wheat spindle streak mosaic bymovirus, wheat yellow mosaic bymovirus, wheat yellow mosaic virus, soil-borne wheat yellow mosaic virus.

Wheat spindle streak mosaic virus belongs to the genus *Bymovirus*, family *Potyviridae* (Brunt *et al*. 1996 onwards).

3 DETECTION

3.1 Symptoms

WSSMV transmission occurs primarily in autumn, but also in spring (Carroll *et al*., 1997), with significant infections taking place during cool, wet autumn periods. Often large areas of a field may be affected (Stromberg 2002) (Fig. 1 and 2), due to the uniform distribution of *P. graminis*, and that WSSMV infection sites tend to follow the lower, wetter areas of the field (Bowden 2005).

Figure 1 Infection by WSSMV in a wheat field (Source: (http://www.uky.edu/Agriculture/IPM/scoutinfo/wheat/disease/wssm/wssm.htm).

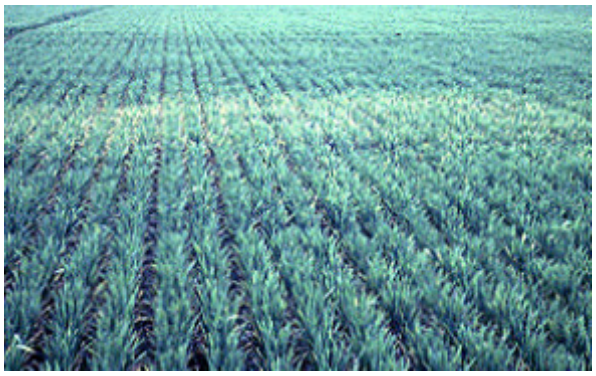

Figure 2 Wheat field showing a large central patch of the crop affected by WSSMV (© Erik L. Stromberg, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech).

Symptoms only appear in early spring, right after green-up (Bowden 2005), regardless of when plants have become infected (Carroll *et al*., 1997), but please note that the earlier in life the wheat is infected, the more severe the symptom expression (Stromberg 2002). The virus requires temperatures below 17°C for symptom development (Lommel *et al*., 1986). If temperatures exceed 15°C infected plants will fail to develop mosaic symptoms, leaving little visual evidence of the disease (Carroll *et al*., 1997; Smith 2005), although symptoms may persist on older leaves (Bowden 2005).

In early spring, the symptoms of WSSMV appear as yellow to light green streaks or dashes on a dark green background, in young leaves. The discontinuous streaks are usually 3 to 6.5 mm long, and oriented parallel to leaf veins and taper at the ends to form chlorotic spindles (Bowden 2005; Smith 2005; Tenuta

and Johnson 2003; Vrandečić *et al.,* 2015) (Fig. 3 to 6). WSSMV is most easily diagnosed by symptoms when the chlorotic spindle shaped lesions appear on the upper leaves of the plant prior to heading (Smith 2005).

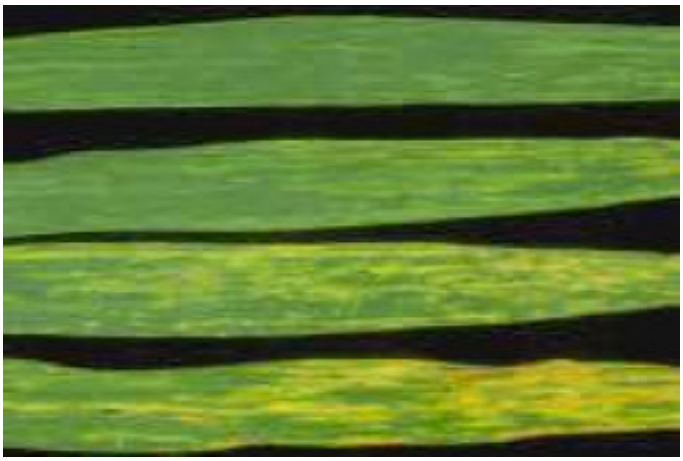

Figure 3 WSSMV symptoms: yellow to light green streaks or dashes 3 mm to 6.5 mm long oriented parallel to the leaf veins (© Robert L. Bowden, Kansas State University).

Figure 4 WSSMV produces yellow streaks running parallel to the leaf veins (Courtesy of Gary Bergstrom, © Queen's Printer for Ontario).

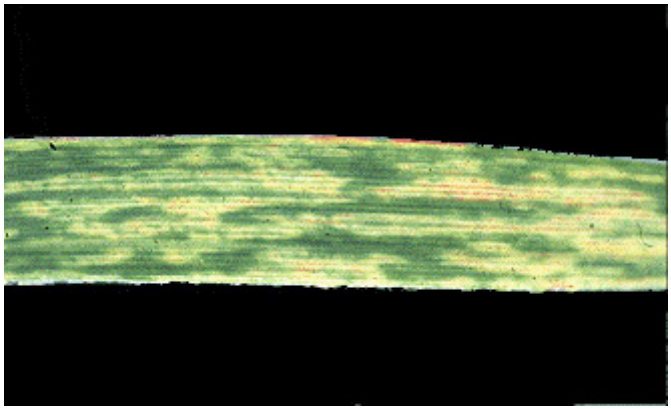

Figure 5 Wheat leaf with spindle streak symptoms of WSSMV infection (© Erik L. Stromberg, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech).

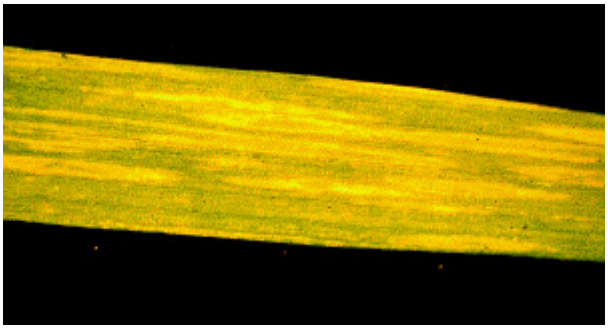

Figure 6 Symptoms of advanced WSSMV infection, showing well-developed short light green to yellow streaks running parallel with the veins in leaves (Source: http://www.uky.edu/Agriculture/IPM/scoutinfo/wheat/disease/wssm/wssm.htm).

During cool spring conditions, and as plants mature, the spindle-shaped lesions and the chlorotic streaks on older leaves coalesce and become necrotic (Smith 2005; Stromberg 2002). The older leaves look mottled with yellow and green blotches. Spindle shaped lesions will continue to develop throughout spring in the upper new leaves if temperatures remain cool (Smith 2005). Warmer temperatures may prevent symptom development on newly expanded leaves, giving the appearance that plants have outgrown the disease, with symptoms confined to older, lower leaves (Smith 2005).

WSSMV can also cause stunting, reduced tillering, smaller heads and less seed per head than healthy plants, although the kernels produced by infected plants are not shrivelled or light in weight (Bowden 2005; Smith 2005; Stromberg 2002; Tenuta and Johnson 2003) (Fig. 7 and 8).

Figure 7 Stunting with WSSMV in susceptible (right) wheat varieties (© Alabama Cooperative Extension System).

Figure 8 Reduction in tillering due to virus infection in a wheat field with a cultivar susceptible (left) (© Erik L. Stromberg, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech).

3.2 Viruses causing similar symptoms in wheat and rye

3.2.1 Not reported in Australia

Wheat yellow mosaic virus

Symptoms caused by wheat yellow mosaic virus (WYMV) on wheat are very similar to those caused by WSSMV (Clover and Henry 1999). These viruses can only be accurately differentiated using molecular tests. Like WSSMV, WYMV is also vectored by *P. graminis* and is likely to have a similar life cycle to WSSMV (Liu *et al*., 2016).

Soil-borne wheat mosaic virus

Soil-borne wheat mosaic virus (SBWMV) (synonym wheat soil-borne mosaic virus) also causes similar symptoms to WSSMV on wheat. As both viruses are vectored by *P. graminis* they share a similar life cycle and distribution pattern in the field (Bowden 2005; Tenuta and Johnson 2003). Both SBWMV and WSSMV may be present in the same field (Tenuta and Johnson 2003), in which case, many plants can be found to be infected with both viruses (Bowden 2005).

Although both viruses are vectored by the same vector, WSSMV and SBWMV are unrelated taxonomically (Bowden 2005; Linker *et al*., 1994; Smith, 2005). WSSMV belongs to the genus *Bymovirus*,

and SBWMV belongs to the genus *Furovirus*. Several other wheat mosaic virus species from the genus *Furovirus*, are likely to share symptomology with WSSMV, these include Chinese wheat mosaic virus and Japanese soil-borne wheat mosaic virus (ICTV Online). Also, WSSMV infects only wheat and rye, SBWMV also infects barley (*Hordeum vulgare*) (Linker *et al*., 1994).

As with WSSMV, symptoms of SBWMV infection often appear in low, wet areas, but may also cover all or most of a field if the vector is widely distributed (Fig. 9) (Linker *et al*., 1994). Symptoms of WSSMV are usually visible prior to symptoms associated with SBWMV, which typically appear in early spring right after green-up (Bowden 2005). The symptoms of SBWMV are most obvious in the early spring months (Linker *et al*., 1994), and as with WSSMV, symptoms will not be expressed in late spring when conditions are warmer (Linker *et al*., 1994; Smith 2005).

Figure 9 SBWMV field disease pattern (left), and patch of SBWMV affected winter wheat (right) (Left; Source: http://www.plantpathology.tamu.edu/appel/vwheat3.htm, right; © Department for Environment, Food and Rural Affairs).

The archetypal symptoms of WSSMV are the yellow-to-light green tapered streaks running parallel to the leaf veins. In contrast, SBWMV typically has a mosaic of green islands or blotches occurring on the leaves, ranging from mild green to a prominent yellow that are longer than they are wide, against a yellow background (Fig. 10 and 11) (Bowden 2005; Linker *et al*., 1994; Smith 2005; Tenuta and Johnson 2003). It must be noted that plants severely infected with WSSMV resemble infection by SBWMV as they may develop mottled leaves (Bowden 2005), and that both WSSMV and SBWMV cause plant stunting (Fig. 12) (Linker *et al*., 1994).

Figure 10 SBWMV symptoms on wheat (left), and close-up of SBWMV affected plants (© Department for Environment, Food and Rural Affairs).

Figure 11 In each photograph, a healthy plant (left) is compared to a plant showing symptoms of SBWMV infection (right).

Figure 12 Severe stunting of wheat caused by SBWMV (© Ohio State University).

3.2.2 Reported in Australia

Wheat streak mosaic virus

Wheat streak mosaic virus (WSMV) is a virus which belongs to the genus *Tritimovirus* (family *Potyviridae*) and is transmitted by the wheat leaf curl mite (*Aceria tulipae*). This virus produces symptoms similar to WSSMV infection, stunted plants with mottled green and yellow streaks extending the length of the leaf (Fig. 14 and 15). Plants can also exhibit rolling or curling of the youngest leaves due to damage by the mite vector. Symptoms of WSMV typically appear in the spring (Stromberg 2002). Unlike WSSMV, WSMV field infection is not evenly distributed, infection is usually located near the field edges, and infected plants continue to show symptoms throughout the increasingly warm spring weather (Smith 2005; Stromberg 2002). WSMV has been reported in Australia (Ellis *et al.,* 2003; Coutts *et al.,* 2008).

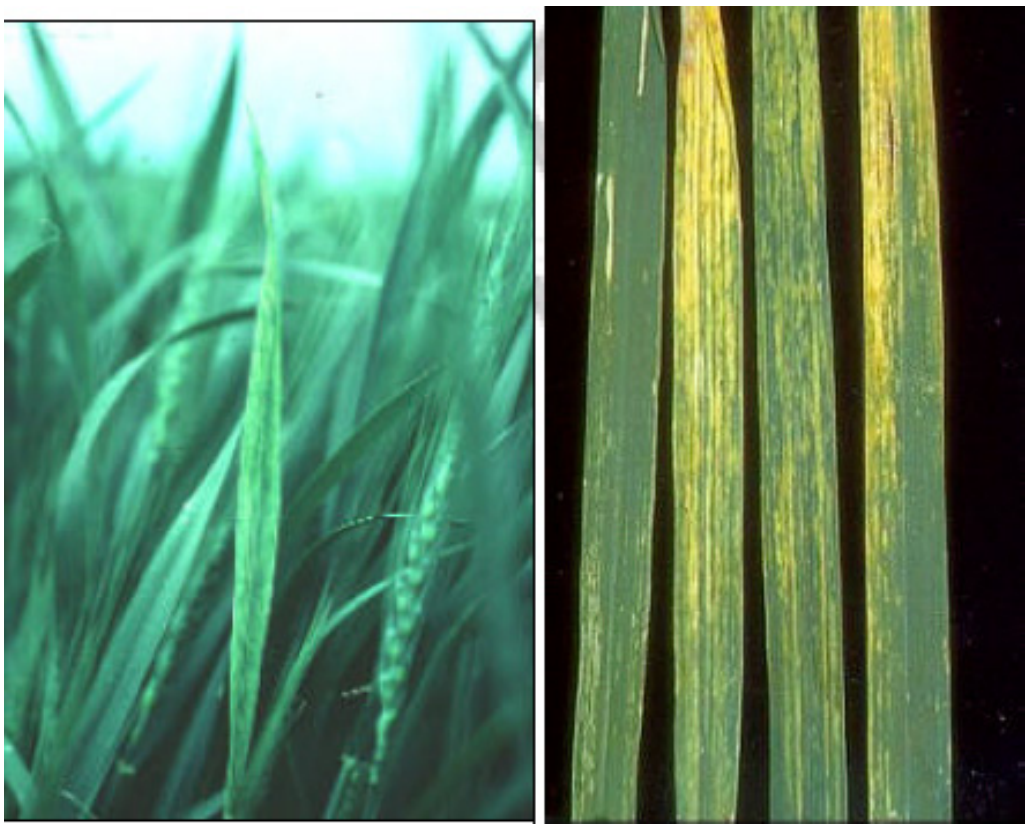

Figure 14 Streak mosaic pattern of WSMV (Left; © 2000 by the University of Nebraska, right; http://www.oznet.ksu.edu/path-ext/Alerts/2002%20alerts/alert04.asp).

Figure 15 As the wheat crop develops, plants affected with WSMV are typically severely stunted with yellow mottled and streaked leaves. (© Erik L. Stromberg, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech).

3.3 Sampling

3.3.1 Plant material

Collect leaves and roots from plants suspected of WSSMV infection. Wash roots clean of soil under fast-running tap water. Store plant material at 4°C or at -20°C (Gitton *et al*., 1999; Kingsnorth *et al*., 2003). Please note that fully expanded young leaves at the top of the plant with suspect symptoms, are preferred to be collected and used.

3.3.2 Soil samples

Collect soil samples in a "W" pattern across the field. Take a 1-cup sample of soil every ten metres. Soil samples can be pooled. To increase the likelihood of detecting virulent *P. graminis*, include soil samples collected from the wettest area of the paddock.

To recover WSSMV from the soil samples, rear *Triticum aestivum* seedlings in the suspect-virus-infected soil (Clover and Henry, 1999). Incubate two-week old seedlings of *T. aestivum* in a slurry of 40 g of infected soil at 20°C for 10-14 days. After incubation, transplant into sterile sand to give a 1:9 dilution of infected soil:sand. Grow the plants for a further 3 weeks at 20°C, then decrease the temperature to 10°C. Record plant symptoms. Take leaf samples monthly. Store plant material at 4°C or at -20°C.

Currently, there are no soil extraction protocols for extracting WSSMV directly from soil.

4 IDENTIFICATION

4.1 Morphological characteristics

The long flexuous filamentous WSSMV particles (Fig. 16) are 16 nm wide with no clear modal length. Length ranges from 300-2000 nm, with the modal length probably 700-1000 nm (Brunt *et al*., 1996 onwards; Jones, 2004; Lommel *et al*., 1986). Distinctive pinwheel inclusion bodies are produced in infected plant tissue (Lommel *et al*., 1986). Virions are found in the cytoplasm of leaves, roots, mesophyll and vascular parenchyma of WSSMV-infected plants. Infection by WSSMV causes the chloroplasts and mitochondria to swell, resulting in disruption of these organelles (Brunt *et al*., 1996).

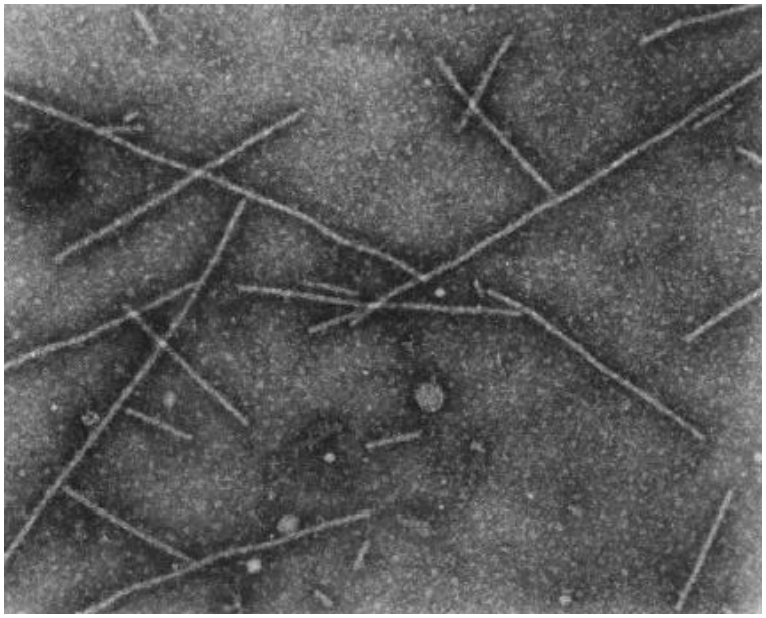

Figure 16 Electron micrograph of *Bymovirus* particle (© Brunt *et al*. 1996 onwards).

4.2 Molecular properties

The RT-PCR protocols published by Gitton *et al*. (1999) and Clover and Henry (1999) are used for the detection of WSSMV (Table 1). Another useful resource for WSSMV detection is the draft NDP for *Bymovirus* (Zheng and Rodoni, Unpublished) which contains a *Bymovirus* generic PCR test which detects WSSMV and other wheat mosaic viruses.

4.2.1 RNA extraction from plant tissue using QIAGEN RNeasy™ Plant Mini Kit

Reagents

- Ethanol 100% (room temperature)
- QIAGEN RNeasy™ Plant Mini Kit reagents (Or similar RNA extraction kit)
- BioRad Quantum Prep® Freeze 'N Squeeze DNA gel Extraction Spin Columns. (Or similar kit for purification of DNA including PCR products)
- PVP-40 (if using the modified protocol)
- 20% (w/v) Sarkosyl (if using the modified protocol by MacKenzie *et al.,* 1997)
- 2 M sodium acetate, pH 5 (if using the modified protocol by MacKenzie *et al.,* 1997) For 100 ml: Sodium acetate 16.406 g
- Adjust pH to 5 using acetic acid

Method

The QIAGEN RNeasy™ Plant Mini kit provides two extraction buffers, the RLT and the RLC, which contain guanidine isothiocyanate (GITC) and guanidine hydrochloride, respectively. In most cases, the buffer RLT is the lysis buffer of choice due to the greater cell disruption and denaturation properties of GITC.

If using the QIAGEN RNeasy Plant Mini Kit, add 10 µl of β-Mercaptoethanol to 1 ml extraction RLT buffer. Follow the manufacturer's instructions from this point forward.

4.2.2 Detection of WSSMV using one-step RT-PCR

For reliable detection of WSSMV, total RNA extracts are subjected to three RT-PCR tests, as outlined below. Primer sequences and PCR cycling conditions are listed in Table 1.

1. Primer pair Fw4 (F)/Rw4 (R). This primer pair amplifies a region of RNA1 on the WSSMV genome, from bases 730 to 1186 on the reference sequence X73883, generating an amplicon of 457 bp.

2. Primer pair WSSMVF (F)/Oligo-d(T)Not1 (R). This primer pair amplifies a region on the WSSMV coat protein gene on RNA1, generating a 982 bp fragment (Clover and Henry, 1999).

3. The Internal control primer pair MDH-H968 (F)/MDH-C1163 amplifies a 196 bp region of the plant mRNA encoding malate dehydrogenase (MDH) gene (Nassuth *et al*., 2000). This plant gene is highly conserved and the internal PCR control is used to determine the quality of the RNA extract, and whether the RNA extract is PCR competent. The internal control PCR is particularly important to rule out false negatives.

4. Alternatively, NAD5 (Thompson *et al*., 2003) can be used as the internal control.

5. Primer pair BymoLZF (F)/BymoLZR (R). This primer pair amplifies a highly conserved region within RNA1 of *Bymovirus* species (approximately 418bp) (NDP Bymovirus).

Table 1 Primers required for the detection of WSSMV

Primer	Sequence (5'-3')	PCR Cycling Conditions	Size (bp)	Region
Fw4[1]	AAGGAAATAAATACCGCCCCAG	1 cycle [48°C 30 min] 1 cycle [94°C 4 min] 25 cycles [94°C 1 min, 56°C 1 min, 72°C 1.5 min] 1 cycle [72°C 10 min]	457	RNA1
Rw4[1]	TCATACCCGACTCTTCCAGCAC			
WSSMVF[2]	CAGCAA CCA AAGTYRCAGCAA C	1 cycle [48°C 30 min] 1 cycle [94°C 2 min] 10 cycles [94°C 30 s, 56°C 30 s, 68°C 1 min] 25 cycles [94°C 30 s, 56°C 30 s, 68°C 2 min] 1 cycle [68°C 7 min]	982	Coat Protein RNA1
Oligo-d(T)Not1[2]	CAA TTC GCG GCC GC(T)			
MDH- H968[3]	GCA TCT GTG GTT CTT GCA GG	1 cycle [48°C 45 min] 1 cycle [94°C 2 min] 35 cycles [92°C 30 secs, 54°C 30 secs, 72°C 1 min], 1 cycle [72°C 5 mins]	192	MDH gene
MDH-C1163[3]	CCT TTG AGT CCA CAA GCC AA			
AtropaNad4 2.1a[4]	5'-GGA CTC CTG ACG TAT ACG AAG GAT C-3'	1 cycle [48°C 45 min] 1 cycle [94°C 2 min] 35 cycles [94°C 30 secs, 50°C 30 secs, 72°C 30 min], 1 cycle [72°C 5 mins]	188	NADH dehydrogenase ND2 subunit
AtropaNad2.2b[4]	5'-AGC AAT GAG ATT CCC CAA TAT CAT-3'			
BymoLZF[5]	5'-CIC CIC AYA CIG TIG GHA T-3'	1 cycle [48oC 30 min], 1 cycle [94oC 2 min], 35 cycles [94oC 30 sec, 48oC 30 sec, 72oC 30 sec], 1 cycle [72oC 5 min]	418bp*	RNA1
BymoLZR[5]	5'-AAI TTI TTI TCA TCR CCR TTG-3'			

[1]Gitton *et al*., 1999; [2]Clover and Henry, 1999; [3]Nassuth *et al*., 2000; [4]Thompson *et al*., 2003 [5]NDP for *Bymovirus* *Size of band dependent on virus strain.

RT-PCR reagents

One-step RT-PCR kit (Invitrogen® SuperScript™ One-Step RT-PCR with Platinum® Taq, Catalogue No. 12574-026), is recommended, however already validated RT-PCR kits within individual laboratories can also be used. The PCR mix are shown in Table 2.

Table 2 One-step RT-PCR conditions using SuperScript III One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (Invitrogen) (Reaction volume: 25 µL)

PCR reagents	Volume per reaction (25 µL)
Sterile distilled H_2O	7.25
2X master mix	12.50
5 µM forward primer	1.25
5 µM reverse primer	1.25
10 mg/mL BSA (optional)	1.25
SuperScript III/Platinum Taq Mix	0.50
RNA template	1.0

PCR Controls

- Positive control - RNA extracted from plant tissue infected with WSSMV, alternatively a "plasmid control" that has the target WSSMV sequence cloned into the plasmid (pGEMT). The positive controls can be purchased from commercial suppliers, e.g. DSMZ (Germany) or Agdia (USA) or from overseas researchers.
- Negative plant control - RNA extracted from uninfected plant tissue of the same species as that used for the positive control.
- Negative buffer control - an aliquot of the PCR Master Mix without template.
- The internal control PCR. Failure to produce an amplicon of expected size (196bp/188bp) indicates that either dilution of the RNA extract is required to dilute out inhibitors or re-extraction of RNA from the sample is required.

Other reagents

5 x TBE buffer	**Per 1 litre**
Tris ($C_4H_{11}NO_3$)	54 g
Boric acid (H_3BO_3)	27.5 g
0.5M EDTA ($[CH_2.N(CH_2.COOH).CH_2COONa]_2.2H_2O$) pH 8.0	20 mL

Store at room temperature.

SYBR Safe (Invitrogen)

Use a 1% DNA grade agarose (Invitrogen #10975-035) (w/v) gel made with 0.5TBE solution and stained with SYBR™ DNA Gel Stain (Invitrogen).

1x TE Buffer	***Per 100 mL***
1 M Tris-HCl (pH 8.0)	1 mL
0.5 M EDTA	200 µL

Adjust pH to 8.0± 0.2. Store at room temperature.

6 times loading dye	*Per 100 mL*
1 x TE	10 mL
Glycerol (Sigma 200-289-5)	50 mL
Bromophenol blue (Sigma 263-653-2)	trace (0.2%)

Store at room temperature.

4.2.3 One-step RT-PCR procedure

This method is to be repeated for each set of the three sets of primers used. Use one-step RT-PCR reagents as specified by the manufacturer. Some volumes outlined below may vary depending on the buffer and enzyme concentrations specified by the manufacturer. Ensure that the final volume of the RT-PCR is 25 µL by altering the volume of nuclease-free water accordingly.

1. Label sterile PCR tubes
2. Prepare "Master Mix" on ice in a sterile microcentrifuge tube. The “Master Mix” usually contains buffer, forward and reverse primers, RT/Taq and nuclease-free water. Prepare the “Master Mix” according to the RT/Taq manufacturer’s recommendations. Ensure that the final volume for each reaction is 24 µl. Add 24 µl of Master Mix to each PCR tube.
3. Add 1 µL of template (total RNA extract) to the corresponding labelled PCR tube.
4. Cycle the tubes using the PCR conditions listed in Table 1.
5. Once the PCR has completed PCR cycling, mix 10 µL each reaction with 2 µL of 6x gel loading dye, and load samples onto a 2% agarose gel with ethidium bromide.
6. Electrophorese in 0.5 times TBE at 100V for 45 minutes or until the Bromophenol blue front has migrated halfway down the length of the gel.
7. Visualise and photograph gel on UV transilluminator.

4.2.4 PCR products purification and sequencing

Following the manufacturer’s instructions (QIAquick PCR Purification Kit, Qiagen or similar), the PCR product can be cleaned directly from the RT-PCR tube or excised from the gel. The product along with the appropriate primers can then be sent to a sequencing facility (e.g., Micromon, Monash University) if it cannot be completed on site. The resulting sequences can then be compared to WSMMV isolates on Genbank, including the type species (NC_040508.1 and NC_040507.1).

4.3 Serological Test - ELISA

The ELISA protocol for WSSMV does not cross react with other viruses. Positive material can be obtained from the Australian Agdia Distributor, if appropriate permits are in place.

The protocol, including buffer recipes can be found on the Agdia website (Agdia - ELISA Reagent Set for Wheat spindle streak mosaic virus (WSSMV).

4.3.1 Equipment

- 20, 100 and 1000ul pipettes and tips
- Microcentrifuge and microcentrifuge tubes (2.0mL)
- Container with ice
- Mortar and pestle/ extraction bags (or similar macerating device)
- ELISA plates
- ELISA plate reader.

4.3.2 Reagents

- Coating Antibody (provided by supplier)
- Detecting Conjugate (provided by supplier)
- Controls: positive and negative (provided by supplier – Permit required).
- 5X PBS Buffer
- PBS-Tween Buffer
- Coating Buffer
- ELISA Extraction Buffer
- Conjugation Buffer
- Substrate Buffer

4.3.3 ELISA Protocol and Interpretation of Results

For WSSMV detection using double-antibody sandwich (DAS)-ELISA using the Agdia (Elkhart, USA), including interpretation of results, follow the manufacturer's instructions. For further information: https://orders.agdia.com/agdia-set-wssmv-alkphos-sra-43001

5 CONTACTS FOR FURTHER INFORMATION

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7 REFERENCES

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8 APPENDICES

8.1 Transmission

WSSMV is transmitted by the obligate plasmodiophoraceous vector *P. graminis* (Barr 1979). The subspecies of *P. graminis* that transmits WSSMV requires 3 hours at 15°C for attachment and host penetration to occur, and WSSMV transmission has been reported to occur within 16 days at temperatures ranging from 7.5 to 17.5°C (Cadle-Davidson and Bergstrom, 2004). Environmental fluctuation through thermoperiodism between 5 to 15°C (night-day) stimulates WSSMV transmission (Cadle-Davidson and Bergstrom, 2004). The virus has not been found to be seed transmitted (Jones, 2004), transmitted by grafting, pollen, or contact between plants (Brunt *et al*., 1996 onwards).

WSSMV particles are thought to be present in viruliferous *P. graminis* resting spores and zoospores (Jones, 2004; Kanyuka *et al*., 2003; Ward *et al*., 2005). Driskel *et al*. (2004) produced direct evidence that WSSMV is internalised by *P. graminis*, as WSSMV coat protein was detected in the resting spores using immunofluorescence.

The precise mechanism of virus uptake and transfer is unknown (Rush, 2003), and it is also not known whether WSSMV is able to replicate within *P. graminis*, but indirect evidence that suggests that it does not (Kanyuka *et al*., 2003).

Motile *P. graminis* zoospores are released from resting spores or zoosporangia, and upon contact with a susceptible host, it infects the plant via root hairs or epidermal cells (Jones, 2004; Rush, 2003). If the zoospore is viruliferous, virus particles are introduced into the plant cytoplasm soon after contents of the zoospore are injected into the cell (Rush, 2003), where the virus replicates and initiates disease, becoming systemic, causing symptoms in leaves, and affecting plant growth and yield (Rush, 2003; Ward *et al*., 2005). All viruses vectored by plasmodiophorids, exhibit in vivo transmission (Rush, 2003).

Non-viruliferous *P. graminis* acquires WSSMV when it multiplies inside virus-infected plant cells (Jones, 2004), as the resulting plasmodium will incorporate the virus (Rush, 2003). If this plasmodium develops into a zoosporangium, the secondary zoospores will be viruliferous, and if it develops into a sporosorus, the virus particles will survive inside the resting spores for years (Rush, 2003). When the plant host cell dies and deteriorates, the infected resting spores are released into the surrounding soil, and upon germination, will release motile viruliferous primary zoospores in search of a plant host (Rush, 2003).

In an infected plant, Carroll *et al*. (1997) detected WSSMV in roots one month prior to its detection in leaves, suggesting that virus moves slowly from roots to leaves. They also detected WSSMV in non-symptomatic leaves from two months after sowing, through to crop senescence. It was concluded that WSSMV is acquired and spread by the vector during the majority of the crop cycle (Carroll *et al*., 1997).

9 DIAGNOSTICS PROCEDURES TO SUPPORT SURVEILLANCE

9.1 Introduction

Wheat spindle streak mosaic virus belongs to the genus *Bymovirus*, family *Potyviridae* (Brunt *et al*., 1996; King *et al*., 2011) and primarily infects wheat (*Triticum aestivum*), durum wheat (*T. durum*), triticale (× *Triticosecale*) and rye (*Secale cereale*) Brunt *et al*., 1996 onwards; Jones, 2004, Jezewska and Trzmiel, 2007).

Symptoms often only become visible at temperatures of >17°C and often appear as light green streaks or dashes on a dark green background, in young leaves (Fig 1) (Bowden 2005; Smith 2005).

Figure 1 WSSMV symptoms: yellow to light green streaks or dashes 3 mm to 6.5 mm long oriented parallel to the leaf veins (© Robert L. Bowden, Kansas State University).

Testing for WSSMV includes common laboratory methods such as RT-PCR, ELISA and morphological identification (Table 1). ELISA is recommended in situations where the number of samples are considered very high (+200), RT-PCR can then be undertaken on any samples that return a positive ELISA result. There are currently no in-field diagnostic tests for the detection of WSMMV, however sampling of suspect plants only, but not asymptomatic plants, may reduce the number of samples required for testing in the laboratory.

Table 1 Diagnostic protocols for the detection of WSSMV.

Method	Identification level	Identification Confidence	Deployment (Field/Lab)	Required Time	Throughput (No. of samples)
Morphological (4.1)	To Genus	Low (<90%)	Field/Laboratory	<1 d - >1 d	Low (1-10)
RT-PCR (4.3)	To Species	High (99%+)	Laboratory	<1 week	High (100s)
ELISA (4.2)	To Species	Medium (90-99%)	Laboratory	<1 week	Very High (1000s)

9.2 Sampling

Sampling should only take place in early spring and when temperatures are below 17˚C. Leaves and roots from suspect WSSMV plants should be sampled individually for small numbers of samples or in bulk samples of one leaf per plant with 10 leaves per bulk sample collected. Roots should be washed under fast-running tap water in order to remove all soil (Gitton *et al*., 1999; Kingsnorth *et al*., 2003).

9.3 In Field Tests

There are no in field tests available for WSSMV.

9.4 Laboratory Tests

Laboratory methods for the detection of WSMMV include: Reverse transcription polymerase chain reaction (RT-PCR) and direct DA-ELISA. Both methods, including the instructions to complete testing, can be located in the NDP for WSMMV in sections 4.2 and 4.3 respectively. There is also an RT-PCR and RT-PCR (SYBR) assay developed by Vaïanopoulos *et al*. (2006) (Table 2) that may be utilised, however they have not been validated.

Table 2 RT-PCR and RT-PCR (SYBR) parameters for Vaïanopoulos *et al*. (2006) assays.

Primer	Sequence (5'-3')	PCR Cycling Conditions	Size (bp)	Region
WSSMV1-F	AGCAACCTTAGCGAAGT	1 cycle [48°C 45 min] 1 cycle [94°C 2 min] 38 cycles [94°C 30 sec, 58°C 30 sec, 72°C 30 sec] 1 cycle [72°C 7 min]	200	Coat protein
WSSMV1-R	AGGGACGTGGAACAAAGA			
WSSMVc2-F	GCAACCTTAGCGAAGTCAG	1 cycle [95°C 3 min] 40 cycles [95°C 30 s, 58°C 1 min] Melt Curve 80 cycles [55°C 10 s, 0.5°C increase per cycle]	199	Coat protein
WSSMVc1-R	AGGGACGTGGAACAAAGAAA			

9.5 Acknowledgements

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9.6 References

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