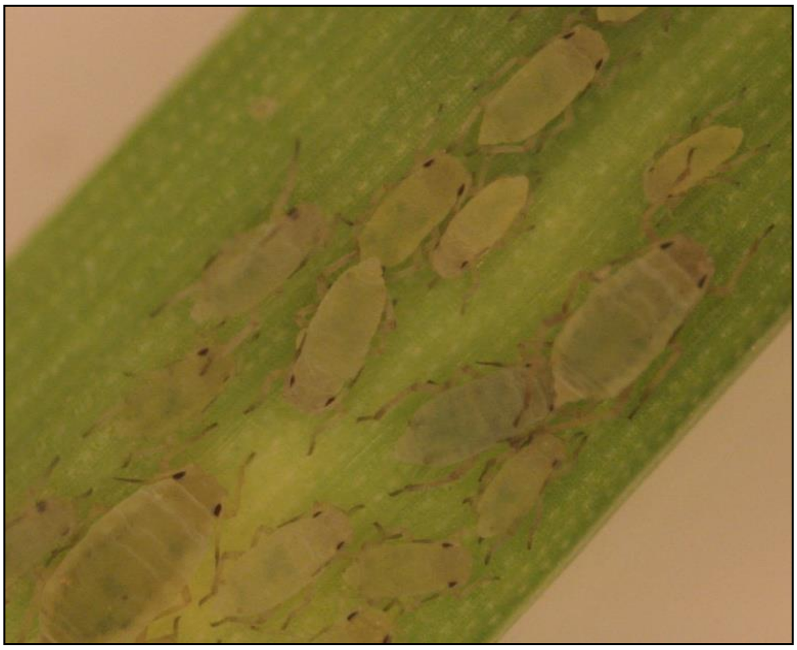
National Diagnostic Protocol

Diuraphis noxia

Russian wheat aphid



NDP 28 V1

***Diuraphis noxia* is now present in Australia. This protocol is being kept as a resource but will not be reviewed or updated.**

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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 *Diuaphis noxia* 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

Russian wheat aphid (*Diuraphis noxia* Kurdjumov) (RWA) is a major pest of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Other members of the Poaceae family that host RWA and for which it is a pest are oats (*Avena sativa* L.), rye (*Secale cereale* L.), Triticale and sorghum (*Sorghum bicolour* (L.) Moench). Russian wheat aphid's host range is limited to grasses in the Poaceae family (see Appendix A).

Toxins injected by RWA into the plant during feeding destroy chlorophyll and prevent carbohydrate formation with heavy infestations killing the plant (Gordh and Headrick 2001). The affected plant is identifiable by the resulting whitish, yellow and red leaf markings and rolling leaves. Late season feeding by RWA, when the wheat head is unfurling, causes the flag leaf to curl around trapping the wheat head and results in incomplete head emergence and grain fill. Russian wheat aphid is a vector of barley yellow dwarf virus, brome mosaic virus and barley stripe mosaic virus.

Russian wheat aphid is approximately 2 mm long pale yellowish green with a fine waxy coating. The siphunculi (also termed cornicles) are very short, rounded, and although present can be difficult to see and often appear to be absent. The RWA's most distinguishing feature and what sets it apart from all other aphids occurring on small grains cereals is an appendage above the cauda, a supracauda, giving the aphid the appearance of having two tails. Winged RWA (alates) are a pale green marginally darker than non-winged (apterous) RWA and have dark patches on the thorax.

Protocols and keys to identify RWA and other aphids infesting cereal crops are widely published. The most authoritative being Blackman and Eastop (2000), Stoetzel (1987), Pike *et al.* (1991) and Aalbersberg *et al.* (1987).

2 TAXONOMIC INFORMATION

Valid name: *Diuraphis noxia* (Kurdjumov) : (Hemiptera: Aphididae: Aphidinae: Macrosiphini)

Synonyms:

- *Brachycolus noxia* Mordwilko
- *Brachycolus noxius* Mordwilko
- *Brachysiphoniella noxius* (Mordvilko ex Kurdjumov)
- *Cavahyalopterus graminearum* Mimeur
- *C. noxius* (Mordvilko)
- *Cuernavaca noxia* (Mordvilko)
- *Cuernavaca noxius*
- *Diuraphis muehlei* (Borner)
- *Diuraphis noxius* (Mordvilko ex Kurdjumov)
- *D. noxia* (Mordvilko)
- *Holcaphis noxius* (Mordvilko ex Kurdjumov)
- *Quernavaea noxia*

Standard common names:

- Russian wheat aphid
- Barley aphid

Source: Hughes R.D. 1996 (revised using Miller *et al.*, 2005)

3 DETECTION

Initial detection of RWA is most likely to be by observation of symptomatic plants. Scouting for infested tillers in wheat and barley crops is the most effective method of detecting RWA under field conditions. Russian wheat aphid is relatively small and a 20X magnification hand lens is essential to locate the leaf damage and aphids when populations are low.

RWA will infest the plant at any growth stage, preferring to feed within the new leaves while leaves are still rolled up, i.e. before the leaf opens. This feeding can prevent the leaf from opening giving the young plant an onion leaf like appearance. Feeding on open leaves causes the leaf to roll inwards around the aphid providing a suitable and protected microclimate. Infested flag leaves that remain unrolled trap the awns and prevent the wheat head from fully emerging and reducing grain fill (Fig. 1).

Initially RWA feeding causes a small light brown blotch that can be confused with damage by other insect and more problematically with symptoms caused by disease (as shown on leaf one, Fig. 2). Leaves infested with RWA develop continuous white, yellowish and red (sometimes described as purplish) streaks along the length of leaf (Fig. 2). The occurrence and intensity of colouration varies, the coloured streaks on young, lightly infested plants often being restricted to the leaf edge and therefore difficult to detect and a hand lens is required for confirmation (Fig. 2B).

Aphid colonies often consist mainly of immature stages on which the key identifying feature of RWA, the supracaudal process, is less well developed so it is important to ensure a number of adults are included in the sample. Alternatively the aphids, complete with host plant material, can be collected into a sealed container for transportation to the laboratory where they can held under controlled conditions to reach maturity for identification. RWA has 5 instars, each identifiable using the number of antennal segments and the ratio between lengths (Aalbersberg *et al.,* 1987).

Figure 1. Wheat awns trapped by flag leaf damaged by Russian wheat aphid (*D. noxia*) feeding damage. Source: Food and Agriculture Organisation, UN.

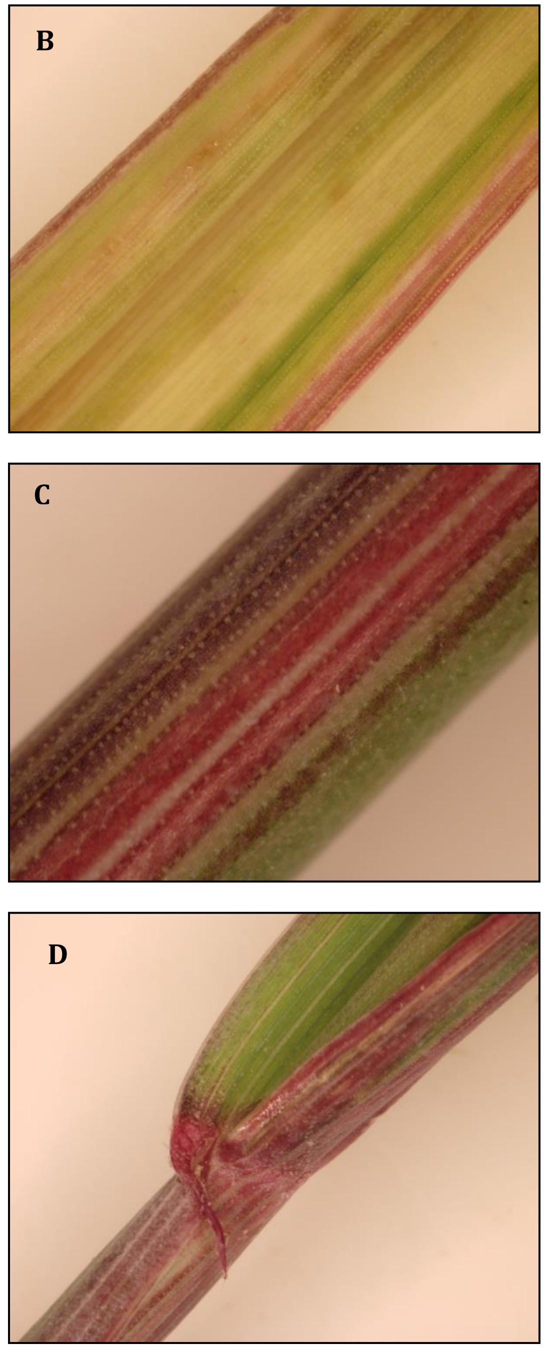

Figure 2 (A, B, C, D). Examples of leaf colour variation and laminar streaking in wheat caused by Russian wheat aphid (*D. noxia*) feeding. Compared to disease (2A Leaf 1). Photos: Nicholas A. 2011 (Oklahoma)

4 IDENTIFICATION

RWA is relatively easy to identify from amongst other cereal and grass aphids currently in Australia owing to its uniquely shaped supracauda process (Figs. 3 and 4.)

RWA are yellowish-green to grey-green with a spindle shaped body of between 1.34 – 2.55 mm in length that is usually covered in a waxy white powder.

The antennae are 6-segmented, the basal and terminal process (unguis), measuring 2-2.2 times longer than the base of the last antennal segment. Apterous RWA have no secondary sensoria on the third antennal segment whereas alata have 4-8 on the third antennal segment and 1-3 on the fourth. Antennal and body hairs are fine and inconspicuous. The last rostral segment is 0.08-0.084 mm long. Cornicles are pale and very short (0.36-0.54 mm), as wide as they are long and almost inconspicuous. The cauda is elongate, 0.114-0.156 mm in length with 4-6 lateral setae. The supracaudal process is present on the dorsum of the eighth abdominal tergite. The supracaudal process is large and conspicuous in the aptera but a shorter knob in alata. Alate RWA are pale but often have a slightly darker green abdomen than aptera and are between 1.332-1.98 mm in length. The eyes and distal third of antennae are dark, the head and thorax having dark patches.

Sources: Stoetzel 1987, Blackman & Eastop 2000, Millar *et al.* 2005.

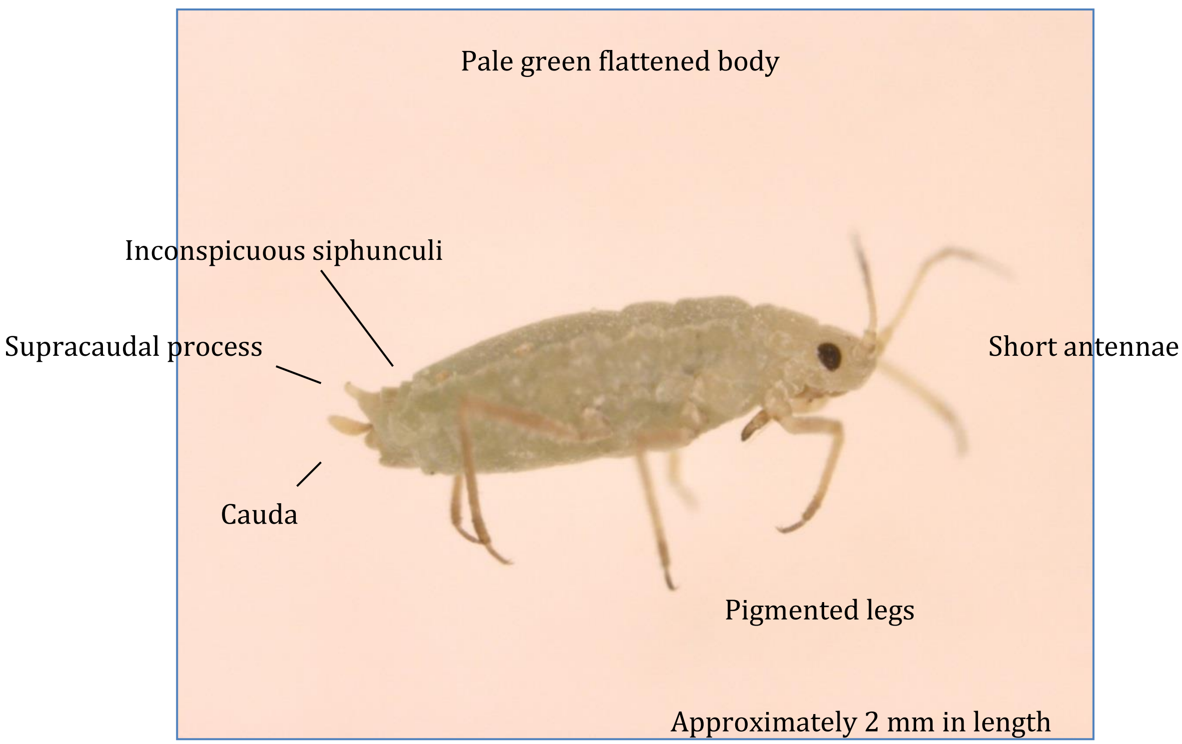

Figure 3. Key identifying features of Russian wheat aphid (*D. noxia).* Photo: Nicholas A. 2011 (Oklahoma).

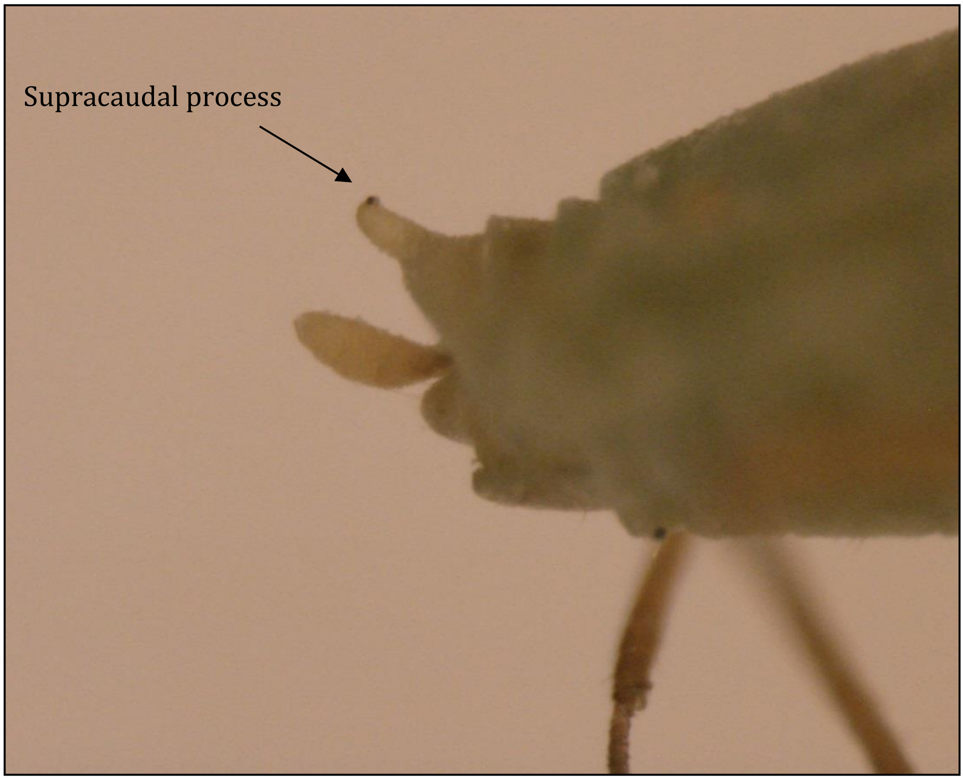

Figure 4. Supracaudal process of Russian wheat aphid (*D. noxia).* Photo: Nicholas A. 2011 (Oklahoma).

Keys for the identification of aphids found infesting cereal crops, including RWA, are widely published. Four of the most authoritative are those by Blackman and Eastop (2000 and 2006), Stoetzel (1987) and Pike *et al.* (1991). The key by Stoetzel is easy to use and well illustrated, while Pike's describes alate *Diuraphis* spp. However both are limited to species found in the US. Other useful keys for the identification of RWA include two papers describing and with keys for RWA instars include Aalbersberg *et al.* (1987), Olsen *et al.* (1993), and a pictorial key to *Diuraphis* spp. by Puterka *et al.* (2010).

Two keys, Puterka et al (2010) (modified) (Appendix B) and Blackman and Eastop (2000) (Appendix C), can be used to quickly identify and distinguished RWA from closely related spp. and other Aphididae present in cereal crops.

Reference specimens of Russian wheat aphid (*Diuraphis noxia*) are held at the NSW DPI Central Coast Primary Industries Centre, Ourimbah (CCPIC), the NSW DPI Scientific Collection Unit, Orange, Australian National Insect Collection (Canberra), DAFWA Insect Collection, Queensland Department of Primary Industries Insect Collect (ref no. 0-142077, 0-142076) and Department of Agriculture (Commonwealth) Sydney Office.

Other reference specimens that are either related to *D. noxia* or occur in Australian cereal crops include *D. frequens, D. mexicana, D. tritici, Rhopalosiphum maidis, R. padi, R. rufiabdominials, Metopolophium dirhodum* and *Sitobion miscathi a*re also held at the CCPIC.

Russian wheat aphid is a relatively small insect making positive identification with a hand lens difficult. For accurate identification using morphological characteristics specimens must be viewed using microscope with a 40-100X magnification. Precise identification is aided by preparing specimens on microscope slides and examining using a compound microscope. Slide mounted specimens should be kept for future reference. A method for making slide specimens is provided in Appendix D.

Updated images are available on the Pest and Disease Image Library (PaDIL) website.
http://www.padil.gov.au/pests-and-diseases/Pest/Main/136095

4.1 Cereal aphids in Australia

Russian wheat aphid can be distinguished from all aphids that currently occur in cereal crops in Australia by the presence of its supracaudal process which is absent in Corn aphid *(Rhopalosiphum maidis),* Wheat/oat aphid, *(Rhopalosiphum padi),* Rice root aphid *(Rhopalosiphum rufiabdominials),* Rose-grain aphid *(Metopolophium dirhodum)* and *Sitobion miscathi*.

4.2 *Diuraphis noxia* related species

As currently defined there 10 species in the genus *Diuraphis* in two recognised subgenera;

Diuraphis sensu stricto containing of two species,
D. mexicana (McVicar Baker) *(previously D. nodulus)* (Fig. 5) and
D. noxia (Kurjumov; Fig. 6);
and the subgenus *Holcaphis* with species
D. agropyronophaga G.-x. Zhang,
D. agrostidis (Muddathir),
D. bromicola (Hille Ris Lambers),
D. calamagrostis (Ossiannilsson),
D. elymophila G.-x.Zhang,
D.frequens (Walker),
D. holci (Hille Ris Lambers), and
D. tritici (Gillette)
(Miller *et al.*, 2005).

The species referred to *D. muehlei* (Börner) in the subgenus *sensu stricto* was found to be a junior synonym of RWA (Miller *et al.* 2005). Species in the subgenus *Diuraphis sensu stricto* differ from those in the subgenus *Holcapaphis* by the presence of a supracaudal on the eighth abdominal tergite and usually the presence of marginal tubercles on abdominal segments II-IV (Miller *et al.* 2005).

There are 12 species in the genus *Diuraphis* of which only three are hosted by wheat and barley. *Diuraphis noxia* (RWA) occurs in both wheat and barley, *D. tritici* in wheat and *D. holci* in barley.

The following are notes and descriptions of the *Diuraphis* species that may be confused with RWA either because they have a supracauda (*D. mexicana*) or because they occur in cereal crops (*D. tritici, D. frequens* and *D. holci*). *Diuraphis. tritici, D. frequens* and *D. holc* do not have a supracauda.

4.2.1 Diuraphis mexicana

Diuraphis mexicana has a supracauda that is a very much smaller triangular (deltoid) conical shaped mount when compared to the RWA's supracauda (which is distinctively pronounced, often described as a fingerlike protrusion). *Diuraphis mexicana* does not occur in domestic crops, its host range being restricted to mountain brome wild grasses. The principal host of *D. mexicana* recorded is *Bromus marginatus,* relatives of which occur in Australia (Appendix A).

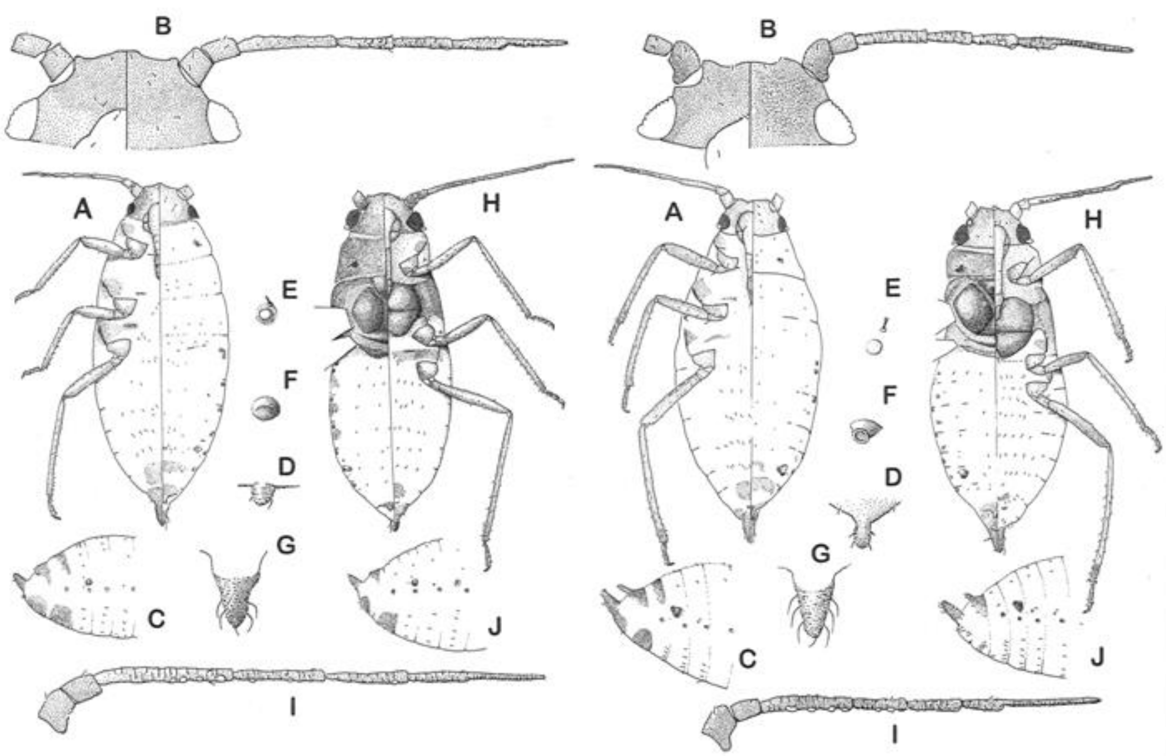

Figure 5. *Diuraphis mexicana*. **Figure 6**. *Diuraphis noxia.*

A, Aptera ventral and dorsal habitus. **B**, Right side, aptera dorsum of head and antennal segments: left side, aptera venter of head and antennal segments I-II. **C**, Aptera lateral abdominal habitus. **D**, Supracaudal process. **E**, Abdominal tubercle. **F**, Cornicle of aptera. **G**, Cauda of aptera. **H**, Alata dorsal and ventral habitus. **I**, Antenna of alata. **J**, Alata lateral abdominal habitus. (Courtesy: Miller *et al*. 2005).

4.2.2 *Diuraphis tritici*

Diuraphis tritici occurs on barley and wheat but is distinctive from RWA by not having a supracauda. *D. tritici*'s rostrum, when held below and parallel with the body is relatively long, reaching to or past the second pair of coxae.

4.2.3 *Diuraphis frequens*

Diuraphis frequens, as for *D. tritci,* is distinctive from RWA in that it does not have a supracauda. Although similar in appearance to *D. tritci*, *D. frequens* can be identified by its shorter rostrum which extends midway between the first and second coxae. This aphid does not occur in cereal crops its host range being restricted to Blue wild rye grass.

4.2.4 *Diuraphis holci*

Diuraphis holci occurs on barley in Europe and is a member of the subgenus *Holcaphis.* As such it can easily be distinguished from RWA by its lack of supracaudal process*. Diuraphis holci* is most similar to *D. frequens* but can be distinguished by longer dorsal head setae that are longer than the widest portion of antennal segment III (Miller *et al.* 2005).

4.3 Non-related species with supracauda in Australia

Any aphid with a supracaudal process that occurs in Australia has the potential to be confused with RWA if picked up in a surveillance program.

4.3.1 *Caveriella species*

Caveriella aegopodii, (Scopoli), the willow carrot aphid, in its apterous form has a significant supracaudal process, although the alate form is described as often rudimentary (Eastop 1966). *Cavariella aegopodii* can readily be distinguished from RWA by its longer siphunculi which are swollen on the apical half, curving to the outside as compared to RWA's short cylindrical siphunculi (Blackman and Eastop 2000, Cottier 1953). *Caveriella aegopodii*, unlike RWA, does not occur on cereal crops but alternates between *Salix* and Umbelliferae in the cooler regions of NSW, Tasmania, Victoria and WA. *Caveriella aspidaphoides, C. pastinacae and C. konoi* all have large supra-cauda but native to the northern hemisphere they are not known to be present in Australia.

4.3.2 *Others species*

Some members of the genera *Galiobium* and *Ossiannilssonia* have a supra-caudal process but are also limited to the northern hemisphere.

4.4 Molecular techniques

Species-specific genetic markers (DNA barcodes) have been determined for Russian wheat aphid and are publically available at GenBank and the Barcode of Life Data Systems (BOLD) websites (refer Appendix Table S1). DNA barcodes identified at this species are genetically monophyletic and exhibit negligible levels of variation across globally sampled populations (Shufran *et al*., 2007; Shufran and Payton, 2011). DNA barcoding can identify Russian Wheat Aphid and distinguish it from other Aphids and *Diuraphis* species at which DNA barcodes are currently available (Shufran and Puterka 2011). Furthermore, DNA barcodes are obtainable from eggs and other stages of the aphid lifecycle (Shufran and Puterka 2011), and are therefore highly useful for identification of the species when samples cannot be identified by other means.

Additional genetic identification methods using Amplified Fragment Length Polymorphisms (AFLP's) provide evidence of geographic structure within the global distribution of Russian wheat aphid (Weiland *et al*. 2008). Information of this type may be used to identify the geographic origins of any new exotic outbreak by the species. AFLP's will be particularly useful to directing remedial biosecurity efforts if it can be shown there is a strong association between virulence of particular source Russian wheat aphid populations and their genetic background.

At the time of publication there are 7 known biotypes distinguished by differences in ability to overcome the plant's natural resistance and in one biotype some resistance to insecticide. This information will be helpful in controlling an outbreak but is subject to change and new information should be sort at the time required.

5 CONTACTS FOR FURTHER INFORMATION

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8 APPENDICES

8.1 Appendix A: Host plants

Table 1. Host plants on which Russian wheat aphid (*Diuraphis noxia*) has been recorded.

Scientific name	**Common name**
Aegilops cylindrica	Jointed goat grass
A. triuncialis	Barbed goat grass
Achnatherum hymenoides	Indian rice grass
Agropyron cristatum *	Crested wheat grass
A. desertorum	Crested wheat grass
A. fragile	Siberian wheat grass
*Andropogon virginicus**	Whisky grass
Aristida oligantha	Prairie three-awn
Avena barbata *	Bearded oats
A. fatua *	Wild oats
A. sativa *	Oats
Bouteloua curtiendula	Side-oats grama
B. gracilis	Blue grama
Bromus erectus *	Meadow brome
B. hordeaceus *	Soft brome
B. inermis *	Smooth brome
B. madritensis *	Spanish brome
B. carinatus *	Mountain brome
B. pectinatus	
B.tectorum *	Drooping brome
B. catharticus *	Prairie grass
Calamovilfa longifolia	Prairie sand reed
Cynodon dactylon *	Bermuda grass
Dactylis glomerata *	Cocksfoot
Digitaria abyssinica *	African couch grass

Scientific name	**Common name**
Eremopyrum triticeum	Annual wheat grass
Festuca arudinacea *	Tall fescue
Hesperostipa comata	Needle-and-thread
Hordeum bulbosum	Bulbous barley
H. murinum *	Barley grass
H. sativa	
H. vulgare *	Barley
Leymus angustus	Altai wild rye
L. cinereus	Basin wild rye
L. racemosus	Mammouth wild rye
Lolium spp. *	Rye grass
L. temulentum *	Darnel rye grass
Nassella viridula	Green needle-grass
Oryza sativa *	Rice
Panicum capillare *	Witch grass
P. virgatum *	Switch grass
Pascopyrum smithii	Western wheat grass
Phalaris canariensis *	Canary grass
P. paradoxa *	
P. aquatica *	
Phleum pretense *	Timothy
Psathyrostachys juncea	Russian wild rye
Pseudoroegneria spicata	Bluebunch wheat grass
Secale cereale *	Rye
S. strictum	Mountain rye

Echinochloa crus-galli *	Barnyard grass	*Setaria pumila* *	Yellow fox tail
Elymus arenarius *	Dune grass	*S. verticillata*	Whorled Pigeon Grass
E. canadensis	Canadian wild rye	*S. italica* *	Green foxtail
E. elymoides	Squirrel tail	*Sporobolus cryptandrus*	Sand drop seed
E. lanceolatus	Streambank wheat grass	*Thinopyrum elongatum* *	Tall wheat grass
E. lanceolatus	Thick-spike wheat grass	*T. intermedium*	Intermediate wheat grass
E. multisetus	Foxtail barley	*T. intermedium* (sub sp.)	Pubescent wheat grass
E. repens *	English couch	*T. pontica*	Rush wheat grass
E. trachycaulus	Slender wheat grass	*Triticum aestivum* *	Bread wheat
Eragrostis cilianensis *	Stink grass	*T. turgidum* *	Durum wheat
E. racemosa	Giant blue wild rice	*X Tritosecale**	Triticale
E. tef *		*Sorghum helepense* (CPC, 2011)*	Johnson grass

* Occurs in Australia. Source: Australian Plant Name Index (APNI) Australian National Botanic Gardens.

Original source: Hughes, 1996. Plant names revised according to USDA ARS National Genetic Resources Program, Germplasm Resources Information Network (GRIN)

[online database] National Germplasm Resources Laboratory Beltsville, Maryland.

URL http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en (27 June 2012).

8.2 Appendix B: *Diuraphis* Key

Key to apterous viviparae Diuraphis species known to occur in cereal crops.

Modified from Puterka *et al.* 2010.

1a. Siphunculi (cornicles) clearly visible and longer than their width. Antennae six segmented and body with few fine hairs **Other Aphididae**

Use Blackman and Eastop (2000) to identify other Aphididae –

1b. Siphunculi barely visible and not longer than their width. Antennae six segmented and body with fine hairs ***Diuraphis* spp.** **2**

2a. Supracaudal process absent or inconspicuous **3**

2b. Supracaudal process present **4**

3a. Rostrum extending midway between the first and second pair of coxae; ultimate rostral segment, usually with black pigment ≈2x longer than wide .

(*D. frequens* included to key *D. tritici*) ....***D. frequens***

3b. Rostrum extending to or past the second pair of coxae; ultimate rostral segment usually with black pigment, ≈3x longer than wide ....***D. tritici***

4a. Supracaudal process short, half as long as wide, deltoid in shape ....***D. mexicana***

4b. Supracaudal process prominent, 2-3x longer than wide, finger-like................. ***D. noxia***

8.3 Appendix C: Aphids Key

Key to aphids occurring in cereal crops (world-wide)

Thirty three aphids have been recorded in wheat, 27 of which can be found in barley*, 16 in oats#), the same key being used for aphids in all cereal crops. Source: Blackman and Eastop 2000.

Note: Blackman and Eastop (2000) include figures to aid identification.

1.	Terminal process longer than base of last antennal segment	2
	Terminal process shorter than base of last antennal segment	19
2.	Dorsal body hairs partly or mainly long and spine-like	3
	Dorsal body hairs short, or if long then fine	5
3.	Cauda with a knob-like apex. Terminal process 2-2.8 times longer than base of last antennal segment	*Sipha flava*
	Cauda broadly rounded in dorsal view. Terminal process 1.3- 2.2 times longer than base of last antennal segment	4
4.	Dorsal abdomen uniformly shiny dark brown or black. Terminal process 1.3-2.2 times longer than base of last antennal segment	*Sipha maydis*
	Dorsal abdomen yellowish brown with some dark markings pleurally, leaving pale sides and a pale spinal stripe. Terminal process 1.6-2.2 times longer than base of last antennal segment	*Sipha elegans*
5.	Siphunculi very small, much shorter than cauda; either papilliform or short rounded cones no longer than their basal widths	6
	Siphunculi tubular, similar in length to or longer than cauda	7
6.	Eighth abdominal tergite with a posteriorly projecting process above the cauda. Terminal process 1.6-2.3 times longer than base of antennal segment	***Diuraphis noxia***
	Eighth abdominal tergite without a supracaudal process. Terminal process less than 1.5 times longer than base of last antennal segment	*Diuraphis tritici*

7.	Siphunculi darker than body (even if rather pale there is still a clear difference in pigmentation between body and bases of siphunculi).	8
	Siphunculi as pale as, or dark only at extreme apices	17
8.	Siphunculi short, less than half as long as the distance between their bases.	9
	Siphunculi more than half as the distance between their bases	13
9.	Cauda long and pale with 4 hairs placed at midlength (no subapical hairs)	*Hysteroneura setariae*
	Cauda dark and rather short	10
10.	Antennae 5-segmented, bearing long, fine hairs, many of which are more than 2 times longer than diameter of antennae segment III.	*Rhopalosiphum rufiabdominalis*
	Antennae 6-segmented, with shorter hairs, mostly shorter than diameter of antennal segment III.	11
11.	Body rather elongate. Terminal process less than 2.5 times longer than base of last antennal segment.	*Rhopalosiphum maidis*
	Body ovate. Terminal process more than 2.5 times longer than base of last antennal segment.	12
12.	Longest hairs on antennal segment III clearly longer than diameter of segment. Posterior abdominal tergites with long pointed hairs similar in length to caudal hairs.	*Rhopalosiphum padiformis*
	Longest hairs on antennal segment III clearly shorter than diameter of segment. Posterior abdominal tergites with mostly short, blunt hairs, clearly shorter than caudal hairs.	*Rhopalosiphum padi*
13.	Siphunculi clavate, without any subapical reticulation.	*Carolinaia rhois*
	Siphunculi tapering, with a subapical zone of polygonal reticulation.	14
14.	Cauda and siphunculi both black.	*Sitobion graminis*
	Cauda pale, or if dusky then much paler than siphunculi	15

15. Siphunculi 1.75-2.25 times longer than cauda, which has a rounded apex. Antennal segments III and IV often paler than more distal segments.	*Sitobion fragariae*
Siphunculi 1.1-1.9 times longer than cauda, which has a more pointed apex. Antennae usually uniformly dark.	16
16. Siphunculi more than 1.4 times longer than cauda, which has a rounded apex. Antennal segments III and IV often paler than more distal segments.	*Sitobion miscanthi*
Siphunculi less than 1.4 times longer than cauda. Hind tarsus segment II more than 1.25 times longer than last segment of rostrum.	*Sitobion avenae*
17. Antennae shorter than the distance from frons to bases of siphunculi. Siphunculi about half as long as the distance between their bases, and usually with dark apices.	*Schizaphis graminum*
Antennae longer than distance from frons to bases of siphunculi. Length of siphunculi more than 0.75 of distance between their bases.	18
18. Each antennal segment pale at base and dusky at apex. Last segment of rostrum less than 0.71 times as long as hind tarsus segment II. (Alatae without dorsal abdominal pigmentation)	*Metopolophium dirhodum*
Antennae progressively darker from base to apex. Last segment of rostrum more than 0.71 times as long as hind tarsus segment II. (Alatae with a segmental pattern of dark markings on dorsal abdomen)	*Metopolophium festucae cerealium*
19. Siphunculi present as pores on low cones.	20
Siphunculi absent.	24
20. Total antennal length 0.33 or more of body length. Tarsi 2-segmented.	*Anoecia* sp.
Antennae very short, less than 0.2 of body length. Tarsi 1-segmented	21
21. Last segment of rostrum less than 0.14 mm long, and less than 1.8 times longer than hind tarsus. Disc of anal plate with some very small hairs in addition to very large ones.	*Tetraneura nigriabdominalis*

Last segment of rostrum more than 0.15 mm long, more than 1.8 times longer than hind tarsus. Disc of anal plate with large hairs only.	22
22. Last segment of rostrum 0.20-0.26 mm long. Antennae and dorsal abdomen densely covered with small hairs. All abdominal wax glands small, and arranged singly.	*Tetraneura radicicoli*
Last segment of rostrum 0.15-0.23 mm long. Antennae and dorsal abdomen sparsely hairy. Abdominal wax glands mainly consisting of multifaceted plates.	23
23. Wax pore-plates (ventrolaterally on each segment) each consisting of a ring of cells around an undivided central area. Last segment of rostrum 0.15-0.19 mm long, less than 2.4 times longer than hind tarsus. Hind tibiae 5-7 times longer than their maximum diameter.	*Tetraneura ulmi*
Wax pore-plates with central area divided or partly divided. Last segment of rostrum 0.18-0.23 mm long, more than 2.3 times longer than hind tarsus. Hind tibiae 3-6 times longer than their maximum diameter, but only more than 5 times longer in alatiform apterae with small compound eyes and 6-segmented antennae.	*Tetraneura africana*
24. Anal plate displaced dorsally.	25
Anal plate in normal ventral position.	27
25. Pronotum sclerotized and pigmented; parallel-sided in unmounted specimens.	*Baizongia pistaceae*
Pronotum weakly sclerotized, continuing curve of body in dorsal view.	26
26. Anal plate clothed with numerous short hairs, but no long ones.	*Geoica utricularia*
Anal plate with long hairs in irregular longitudinal rows, short hairs being confined to the region close to the anus.	*Geoica lucifuga*
27. Body spindle-shaped, about twice as long as wide. Antennae very short, less than 0.17 of body length.	*Aploneura lentisci*
Body rounded or oval in dorsal view. Antennae more than 0.2 of body length.	28

28. Body dorsoventrally flattened. Antennae 6-segmented. Eyes usually with many facets.	*Paracletus cimiciformis*
Body not flattened dorsoventrally, rather globose. Antennae 5- or 6-segmented. Eyes usually with 3 facets.	29
29. Base of last antennal segment clearly longer than penultimate segment. Large wax pore-plates often present, at least on posterior abdominal segments.	*Pemphigus* sp(p)
Base of last antennal segment similar in length to penultimate segment. Wax pore-plates absent.	30
30. Rhinarium on antennal segment V very large, occupying an area at least 4-5 times larger than that occupied by the rhinarium on	*Forda formicaria*
- Rhinarium on antennal segment V not much larger than that on IV.	31
31. Hairs on antennal segment III very short and blunt, less than 0.25 of diameter of segment and barely visible in unmounted specimens. Body hairs also mainly very short and inconspicuous.	*Forda marginata*
Hairs on antennal segment III longer, more than 0.5 of diameter of segment.	32
32. Hairs on antennal segment III erect, thick and bristle-like, mainly of length less than diameter of segment.	*Forda hirsute*
Hairs of antennal segment III often longer than diameter of segment, with very finely pointed apices.	*Forda orientalis*

8.4 Appendix D: Storage & Slide preparation

Techniques for sample storage and micro-slides preparation of aphids

Soft bodied insects such, as aphids, should be collected into 75% ethyl alcohol (ethanol) and can be stored for several years. Insects considered a potential exotic species, i.e. not native to Australia, must be transported in a sealed container.

8.4.1 Preservation

Aphids for morphological examination should be preserved in a sealed container filled with 85-90% ethyl alcohol (ethanol). For prolonged storage one part of 75% w/w lactic acid may be added after a few days to every two parts of ethyl alcohol containing the samples. To prevent long term evaporation, individual sample containers should be stored in a sealed glass jar containing cotton wool soaked in ethyl alcohol. For long term specimen curation, aphids must be mounted on microscope slides using a permanent mounting medium such as Canada Balsam or Euparal.

8.4.2 Slide mounting

Maceration to remove the soft tissue in preparation for slide mounting.

- Using a dry heater block (or water bath at near boiling point), with specimens in glass container heat the specimens in 95% ethyl alcohol for 1-2 minutes.
- Carefully pierce the side of the body wall with a micropin to allow fluid transfer.
- Pipette off excess alcohol, add about 1 cm of 10% potassium hydroxide (KOH) solution and simmer for 4-5 minutes.
- Pipette off excess solution and wash the specimens using 3 changes of distilled water allowing them to soak for 10 minutes each time.

To dehydrate and clear the specimens:

- Pipette off the distilled water and add 1 cm of glacial acetic acid and leave for 3 minutes.
- Pipette off the glacial acetic acid and add 1 cm of glacial acetic acid and leave for 3 minutes.
- Add clove oil or terpineol to clear and leave for 10-20 minutes or until the specimens is clear.

Slide mounting

- Place specimen in a drop of Canada balsam on a clean slide and gently arrange the specimen as required.
- Gently place onto the specimen squeezing out any air bubbles and ensuring the specimen remains untwisted.
- Label and dry the slide in an oven at 50°C for about a week.

Source: Blackman and Eastop, 2000.

8.5 Appendix E: Russian Wheat Aphid mitochondrial DNA COI sequence accessions (GenBank) and DNA barcode specimens records (BOLD).

GenBank accession	BOLD process ID	BOLD sample ID	Authors	Title	Journal
EF474345	GBMH6159-09	EF474345	Shufran,K.A., Kirkman,L.R. and Puterka,G.J.	Absence of Mitochondrial DNA Sequence Variation in Russian Wheat Aphid (Hemiptera: Aphididae) Populations Consistent with a Single Introduction into the United States	J. Kans. Entomol. Soc. 80 (4), 319-326 (2007)
EF474346	GBMH2057-07	EF474346			
EF474347	GBMH2056-07	EF474347			
EF474348	GBMH2055-07	EF474348			
EF474349	GBMH2054-07	EF474349			
EF474350	GBMH2053-07	EF474350			
EF474351	GBMH2052-07	EF474351			
EF474352	GBMH2051-07	EF474352			
EF474353	GBMH2050-07	EF474353			
EF474354	GBMH2049-07	EF474354			
EF474355	GBMH2048-07	EF474355			
EF474356	GBMH2047-07	EF474356			
EF474357	GBMH2046-07	EF474357			
EF474358	GBMH2045-07	EF474358			
EF474359	GBMH6158-09	EF474359			
EF474360	GBMH6157-09	EF474360			
EF474361	GBMH6156-09	EF474361			
EF474362	GBMH6155-09	EF474362			
EF474363	GBMH6154-09	EF474363			
EF474364	GBMH2039-07	EF474364			
EF474365	GBMH2038-07	EF474365			
EF474366	GBMH2037-07	EF474366			
EF474367	GBMH2036-07	EF474367			
EF474368	GBMH2035-07	EF474368			
EF474369	GBMH2034-07	EF474369			
EF474370	GBMH2033-07	EF474370			
EF474371	GBMH2032-07	EF474371			
EF474372	GBMH2031-07	EF474372			

8.5. Appendix E (continued):

GenBank accession	BOLD process ID	BOLD sample ID	Authors	Title	Journal
EF474373	GBMH2030-07	EF474373	Shufran,K.A., Kirkman,L.R. and Puterka,G.J.	Absence of Mitochondrial DNA Sequence Variation in Russian Wheat Aphid (Hemiptera: Aphididae) Populations Consistent with a Single Introduction into the United States	J. Kans. Entomol. Soc. 80 (4), 319-326 (2007)
EF474374	GBMH2029-07	EF474374			
EF474375	GBMH2028-07	EF474375			
EF474376	GBMH2027-07	EF474376			
EF474377	GBMH6153-09	EF474377			
EF474378	GBMH6152-09	EF474378			
EF474379	GBMH6151-09	EF474379			
EF474380	GBMH6150-09	EF474380			
EF474381	GBMH6149-09	EF474381			
EF474382	GBMH2021-07	EF474382			
EF474383	GBMH2020-07	EF474383			
EF474384	GBMH2019-07	EF474384			
EF474385	GBMH2018-07	EF474385			
EF474386	GBMH2017-07	EF474386			
EF474387	GBMH2016-07	EF474387			
EF474389	GBMH2014-07	EF474389			
EF474390	GBMH2013-07	EF474390			
EF474391	GBMH2012-07	EF474391			
EF474392	GBMH2011-07	EF474392			
EF474394	GBMH2009-07	EF474394			
EF474395	GBMH6148-09	EF474395			
EF474397	GBMH6146-09	EF474397			
EF474398	GBMH6145-09	EF474398			
EF474399	GBMH6144-09	EF474399			
EF474400	GBMH6143-09	EF474400			
EF474401	GBMH2003-07	EF474401			
EF474402	GBMH2002-07	EF474402			
EF474403	GBMH2001-07	EF474403			

8.5. Appendix E (continued):

GenBank accession	BOLD process ID	BOLD sample ID	Authors	Title	Journal
EF474404	GBMH2000-07	EF474404	Shufran,K.A., Kirkman, L.R. and Puterka,G.J.	Absence of Mitochondrial DNA Sequence Variation in Russian Wheat Aphid (Hemiptera: Aphididae) Populations Consistent with a Single Introduction into the United States	J. Kans. Entomol. Soc. 80 (4), 319-326 (2007)
EF474405	GBMH1999-07	EF474405			
EF474406	GBMH1998-07	EF474406			
EF474407	GBMH1997-07	EF474407			
EF474408	GBMH1996-07	EF474408			
EF474409	GBMH1995-07	EF474409			
EF474410	GBMH1994-07	EF474410			
EF474411	GBMH1993-07	EF474411			
EF474412	GBMH1992-07	EF474412			
EF474413	GBMH1991-07	EF474413			
EF474414	GBMH6142-09	EF474414			
EF474415	GBMH6141-09	EF474415			
EF474416	GBMH6140-09	EF474416			
EF474417	GBMH6139-09	EF474417			
EF474418	GBMH6138-09	EF474418			
EF474419	GBMH1985-07	EF474419			
EF474420	GBMH1984-07	EF474420			
EF474421	GBMH1983-07	EF474421			
EF474422	GBMH1982-07	EF474422			
EF474423	GBMH1981-07	EF474423			
EF474424	GBMH1980-07	EF474424			
EF474425	GBMH1979-07	EF474425			
EF474426	GBMH1978-07	EF474426			
EF474428	GBMH1976-07	EF474428			
EF474429	GBMH1975-07	EF474429			
EF474431	GBMH1973-07	EF474431			

8.5. Appendix E (continued):

GenBank accession	BOLD process ID	BOLD sample ID	Authors	Title	Journal
EU021093	GBMH4527-08	EU021093	Shufran,K.A. and Payton,T.L.	Limited genetic variation within and between Russian wheat aphid (Hemiptera: Aphididae) biotypes in the United States	J. Econ. Entomol. 102 (1), 440-445 (2009)
EU021094	GBMH4526-08	EU021094			
EU021095	GBMH4525-08	EU021095			
EU021096	GBMH4524-08	EU021096			
EU021097	GBMH4523-08	EU021097			
EU021105	GBMH4515-08	EU021105			
EU021106	GBMH4514-08	EU021106			
EU021107	GBMH4513-08	EU021107			
EU021108	GBMH4512-08	EU021108			
EU021109	GBMH4511-08	EU021109			
EU021110	GBMH4510-08	EU021110			
EU021111	GBMH4509-08	EU021111			
EU021112	GBMH4508-08	EU021112			
EU021113	GBMH4507-08	EU021113			
EU021114	GBMH4506-08	EU021114			
EU021115	GBMH4505-08	EU021115			
EU021116	GBMH4504-08	EU021116			
EU021117	GBMH4503-08	EU021117			
EU021118	GBMH4502-08	EU021118			
EU021119	GBMH4501-08	EU021119			
HQ392571	N/A	N/A	Shufran,K.A. and Puterka,G.J.	DNA Barcoding to Identify All Life Stages of Holocyclic Cereal Aphids (Hemiptera: Aphididae) on Wheat and other Poaceae	Ann. Entomol. Soc. Am. 104 (1), 39-42 (2011)
N/A	GMRMM141-14	BIOUG13725-C06	Angela Telfer	N/A	Vouchered: Registered Collection: not published
	GMRMM156-14	BIOUG13725-D09			
	GMRMM157-14	BIOUG13725-D10			
	GMRMM162-14	BIOUG13725-E03			

8.5. Appendix E (continued):

GenBank accession	BOLD process ID	BOLD sample ID	Authors	Title	Journal
N/A	SDRAG423-14	BIOUG15976-B06	Kate Perez	N/A	Vouchered: Registered Collection: not published
	SDRAS606-14	BIOUG16179-A09			
	GMRMM140-14	BIOUG13725-C05	Angela Telfer		
	GMRMM144-14	BIOUG13725-C09			
	GMRMM163-14	BIOUG13725-E04			
	GMRMM167-14	BIOUG13725-E08			
	SDRAG433-14	BIOUG15976-C04	Kate Perez		
	SDRAM242-14	BIOUG16361-A07			
	GMRMI053-14	BIOUG13601-E07	Angela Telfer		
	GMRMM164-14	BIOUG13725-E05			
	SDRAG426-14	BIOUG15976-B09	Kate Perez		