

# National Diagnostic Protocol

*Mayetiola destructor*

Hessian Fly



*NDP 41 V1*

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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/initiatives/national-diagnostic-protocols/>

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 *Mayetiola destructor* 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 project should be sent to:

[sphd@agriculture.gov.au](mailto:sphd@agriculture.gov.au)

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# 1 INTRODUCTION

The Hessian fly (*Mayetiola destructor*) is a tiny fly (or midge) similar in appearance to a mosquito.

All four life stages (egg, larvae, pupa, and adult) may be found on cereal or grass plants. Elongate, cylindrical glossy red eggs are laid within leaf veins. Hatched maggots are pale and cylindrical growing from 0.5 to 4.0 mm long and feed on hidden parts of the plant such as within leaf sheaths. This feeding damage may cause stunting and death of plants. Larvae pupate approximately three weeks after hatching from egg. Pupae may be found within leaf sheaths and at the base of plants between stems or tillers. They are often referred to as the 'flaxseed' stage given its close resemblance. An adult winged midge emerges from the pupa after 6-33 days, with lower temperatures prolonging development. A full life cycle varies between 20 days to 49 months depending on environmental conditions. Up to six generations per year have been reported in favourable environments (McColloch 1923).

Hessian fly may be confused with the Barley stem gall midge, *Mayetiola hordei*, which is also considered a biosecurity threat to Australia's cereal industry. It may also be confused with other midges introduced or native to Australia.

## 1.1 Hosts

Primary host: *Triticum* spp. (wheat)

Secondary hosts: *Agropyron* (wheatgrass), *Hordeum vulgare* (barley), *Secale cereale* (rye), other grasses

*M. destructor* has been recorded from some grass genera (*Aegilops*, *Lolium*, *Elytrigia*, *Bromus*, *Elymus* and some species of *Agropyron*). *Elytrigia repens* [*Elymus repens*] is an alternative host in Europe, and Barnes (1956) suggested that it may have been the original host of Hessian fly. Reproduction on non-Triticeae grass weeds is negligible.

## 2 TAXONOMIC INFORMATION

Taxonomic placement within the order Diptera (flies):

Family: Cecidomyiidae Subfamily: Cecidomyiinae Tribe: Oligotrophini

Genus: *Mayetiola*

Species: *destructor* (Say)

Name: *Mayetiola destructor* (Say) 1817

Synonyms:

- *Cecidomyia destructor* Say 1817
- *Cecidomyia culmicola* Morris 1849
- *Cecidomyia frumentaria* Rondani 1864
- *Chortomyia secalina* (Loew 1858)
- *Mayetiola secalis* Bollow 1950
- *Phytophaga cerealis* Rondani 1843
- *Phytophaga destructor* (Say 1817)
- *Rhabdophaga elymi* Felt, 1909
- *Rhabdophaga occidentalis* Felt, 1908
- *Rhabdophaga pratensis* Felt, 1908

Common name: Hessian fly

## 3 DETECTION

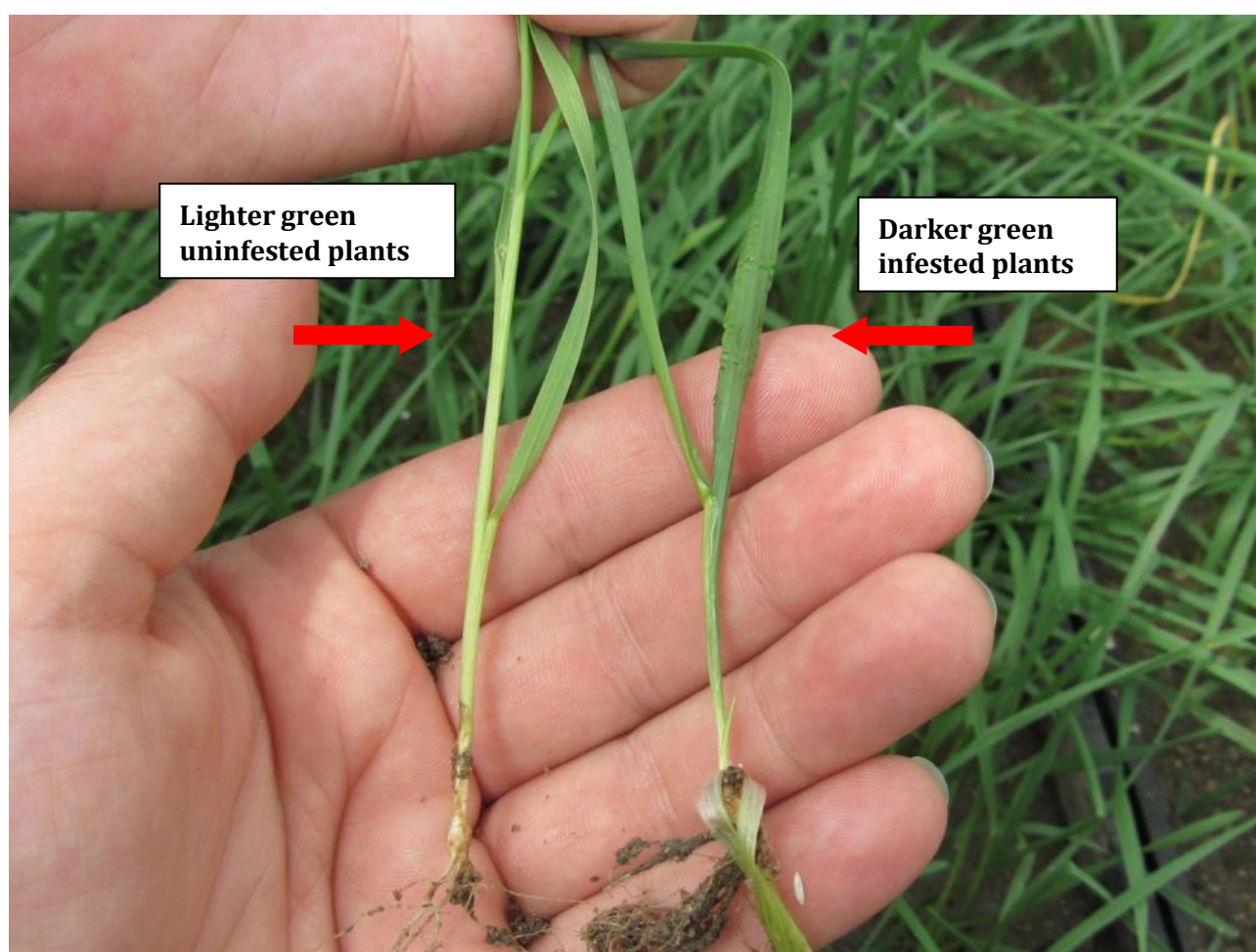
### 3.1 In field

Owing to their small size, cryptic nature and short adult lifespan, the Hessian fly may be difficult to detect in cereal or grass hosts in field situations.

Symptoms of Hessian fly presence may not always be a reliable means of detection, especially where they may be present in very low numbers or dormant within pupae. However, some symptoms are important to be aware of in field situations. These include plants that appear greener and stunted.

#### Patches of darker green plants

Plants infested with Hessian fly, particularly young seedling crops, may appear greener than healthy uninfested plants (Figure 1) as the larva manipulates the plant to take up more nutrients. Patches of plants that are darker green relative to surrounding plants should be inspected for Hessian fly larvae.



**Figure 1.** Wheat plant colour difference with Hessian fly infestation. Kansas State University glasshouse. Photo: Dustin Severtson, Department of Agriculture and Food Western Australia.



## Stunting

Hessian fly infestation in a cereal crop may cause patches of stunted plants where low numbers of female flies have laid eggs in a very localised area. Larvae hidden within the base of plants do not move from the area (i.e. move to other plants). If Hessian fly is suspected, collect plants from the suspect area and adjacent healthy looking plants for comparison of the above and below ground plant parts. Plant should be dug up carefully keeping the roots intact so as not to displace any larvae or pupae.

## 3.2 Plant material

Cereal and grass plant hosts may be inspected for Hessian fly eggs, larvae and pupae. Adult flies may be captured preferably by pheromone traps (see Appendix) or sweep nets with a fine mesh.

### 3.2.1 Eggs

The shiny, oblong reddish eggs may be seen where they are laid along the veins of the upper side of leaves (Figure 2). Severe egg infestation may give the appearance of rust infection. A 10x hand lens will aid in distinguishing eggs from other material such as disease or soil particles.



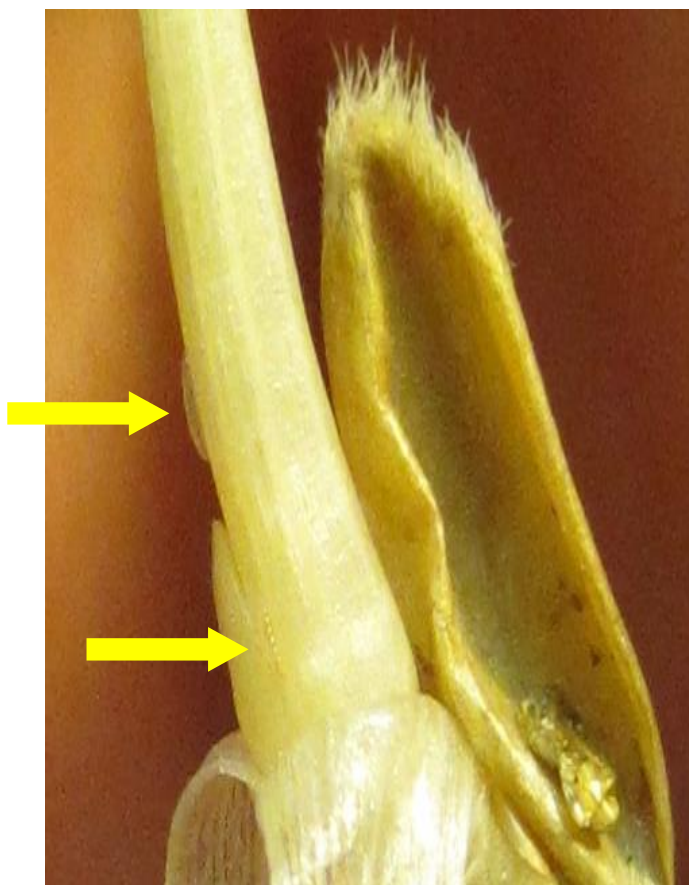
**Figure 2.** Hessian fly egg on the upper surface of a wheat leaf.



### 3.2.2 Larvae and pupae

Suspect plant samples may be inspected by peeling the leaf sheaths down to the crown of the wheat plant where tillers emerge. First instar larvae are very tiny (up to approx. 0.5-1.7 mm long) and can be found on the crown of the plant using a 10x or 20x magnification hand lens. The larvae colour varies from almost transparent to opaque and are easily missed when in low numbers (Figure 3).

Second instar larvae are more noticeable, as they are creamy to white and larger ranging in size from 1.7 to 4 mm. The second instar larvae are immobile and remain within the leaf sheaths at the base of the plant to feed (Figure 4). Third instar larvae are the same, except that they become of the reddish brown (Figure 5) prior to developing into the dark coloured 'flaxseed'-looking pupae (2-6 mm) (Figure 6).



**Figure 3.** Hessian fly first instar larvae are very tiny and cryptic. They crawl to the crown of the plant.



**Figure 4.** Second instar larvae of Hessian fly are larger, pale white and immobile at the base of plants within leaf whorls and between tillers.

Photos: (Kansas, USA) Dustin Severtson, Department of Agriculture and Food Western Australia.



**Figure 5.** Second (pale) and third (red-brown) instars of Hessian fly (10x magnification hand lens). Photo: (Kansas, USA) Dustin Severtson, Department of Agriculture and Food Western Australia.



**Figure 6.** Pupae of Hessian fly are red to dark brown at the base of plants within leaf whorls and between tillers. Photo: (Kansas, USA) Dustin Severtson, Department of Agriculture and Food Western Australia.

## 4 IDENTIFICATION

Molecular markers should be used for identification of *M. destructor* and *M. hordei* males according to: Chen, M.S., Wheeler, S., Davis, H., Jeff Whitworth, R., Knutson, A., Giles, K. L., et al. (2014). Molecular markers for species identification of Hessian fly males caught on sticky pheromone traps. *Journal of Economic Entomology*, 107(3), 1110-1117, doi:10.1603/ec13384.

Many specimens (egg, larva, pupa or adult) should be collected for the molecular test to ensure presence of males.

These methods should be complemented with traditional taxonomic methods based on keys and descriptions already established for identification of *Mayetiola destructor* (Say) adults and larvae.

Identification of *Mayetiola* adults to species level is based principally on microscopic differences in the male genitalia and other characters, and is best undertaken by expert taxonomists. Most species have not been well characterised. There are no native or introduced Diptera of the genus *Mayetiola* currently in Australia. Given this, specialists familiar with the family Cecidomyiidae are unlikely to misidentify the species. However, due to the small size of the family, and lack of *Mayetiola* material available for comparisons, less skilled identifiers may misidentify the genus. The most obvious initial identification available is the damage to crops and the location of the larvae in the host plant. However, correct identification of adult flies will require examination of male genitalia.

### 4.1 Specimen collection and preservation

Specimens can be killed by standard methods (near boiling water or 70% ethanol), and preserved in 70% ethanol. Specimens for DNA analysis should be collected directly into absolute ethanol (adults or larvae) and stored at -20C.

#### 4.1.1 Immediate assessment

High power stereo microscopes may be suitable for species diagnostics of adults using morphological characteristics. Dead adult specimens may be glued to a micropin and observed directly. The dorsal view of the male terminalia should be observed under 160x magnification.

#### 4.1.2 Dissection and slide mounting

Mounting whole specimens requires clearing in 10% KOH for 3-5 minutes, rinsing specimens with distilled water 5-6 times, and slide-mounting using a standard method with a mountant such as Euparal. First instars may not require clearing. Slide mounting of whole specimens may be unnecessary (except for close observations of female genitalia) when using a high power stereo microscope.

Adult male genitalia may be dissected under stereo microscope using a scalpel blade. Gently cut before the last tergite, place directly onto a slide with a drop of Hoyer's medium (which clears the specimen) and orient the male genitalia for a dorsal view (Figures 14, 15). Observe under compound microscope.

## 4.2 Morphological identification

### 4.2.1 Larvae

#### *Family Cecidomyiidae*

- Larval body consists of 13 segments
- Larvae have head capsule with tentorial arms and mouth apparatus apparent

#### *Subfamily Cecidomyiinae*

- Larval anal opening slit-like on ventral side of terminal segment

#### *Tribe Oligotrophini*

- Terminal segment of larvae rounded and with central notch
- Eighth abdominal segment of larvae always bearing two dorsal papillae

#### *Genus Mayetiola*

- Instars of all species reside inside the leaf whorls/sheaths of grasses
- The last instar develops into an adult within the penultimate larval cuticle, without feeding (i.e. “the flaxseed”)
- Larvae have either bifid or hastiform (or pointed) breastbone

### 4.2.2 Adult

#### *Family Cecidomyiidae*

- Elongate antenna 8–24 segmented.
- Antennae ‘simple’ (long, with bead-like segments, often with whorls of hairs)
- Slender-bodied; stilt-legged. Ocelli present, or absent.
- Eyes asymmetric, nearly or quite connected above the antennae.
- The maxillary palps (1–)3–5 segmented; drooping.
- Wing venation consists of a few weak veins (Figure 7)
- Wing veins reaching the margin (2–)3, or 4(–6).
- Wings without a discal cell; without a sub-apical cell; without a closed anal cell.
- The costa extending around the entire wing.
- Sub-costa absent or only dubiously identifiable.
- Wings with the lower calypter much reduced or absent (Watson and Dallwitz, 2003).

### 4.2.3 Identification of *Mayetiola destructor* (Say)

#### **Larvae:** No definitive species-specific characteristics

The first instar is 0.5-1.7 mm long, dorsoventrally flattened at first, but becoming cylindrical with age. The second instar is 1.7-4.0 mm long, unevenly cylindrical and with the posterior end variably tapered. The integument is almost uniformly covered with elongate spicules and the head is directed ventrally beneath the first thoracic segment. While feeding this instar is white, but when feeding ceases it turns brown, becomes hard and its shape may be modified by compression, especially when



crowded. It becomes a puparium within which the third instar, pupa and adult will develop. The third instar develops within the second, is not visible, and does not feed. It is dorsoventrally flattened, becoming cylindrical as the pupal tissues develop. The integument is completely covered with rounded verrucae, except on the anteroventral areas of the ventral segments, which have verrucae tipped with anteriorly directed points. A median, ventral, bifid sternal spatula is present on the prothorax (Gagné and Hatchett, 1989).

**Pupae:** No definitive species-specific characteristics (Figures 5, 6, 11)

- Puparia covered in spiracles

**Adult:**

- Adults 2-4 mm long
- Wing venation reduced (Figures 7, 8, 9)  
Long antennae with a variable number of flagellomeres (usually 2+15 or 2+16 but ranging from 2+14 to 2+18) (Figures 9, 10)
- Female genitalia has a seventh tergite that flares out anteriorly (Figures 12,13)
- Male genitalia has elongate gonostyli and deeply separated hypoproctal lobes (Figures 14, 15)



**Figure 7.** Hessian fly wings showing hairs and venation pattern. Photo: (Kansas, USA) Dustin Severtson, Department of Agriculture and Food Western Australia



**Figure 8.** Hessian fly wings showing hairs and venation pattern. Photo: (Kansas, USA) Dustin Severtson, Department of Agriculture and Food Western Australia



**Figure 9.** Mating Hessian flies showing wing venation and antennae. Photo: Entomology Dept., University of Nebraska



**Figure 10.** Hessian fly antennae showing whorls of hairs. Photo: (Kansas, USA) Dustin Severtson, Department of Agriculture and Food Western Australia

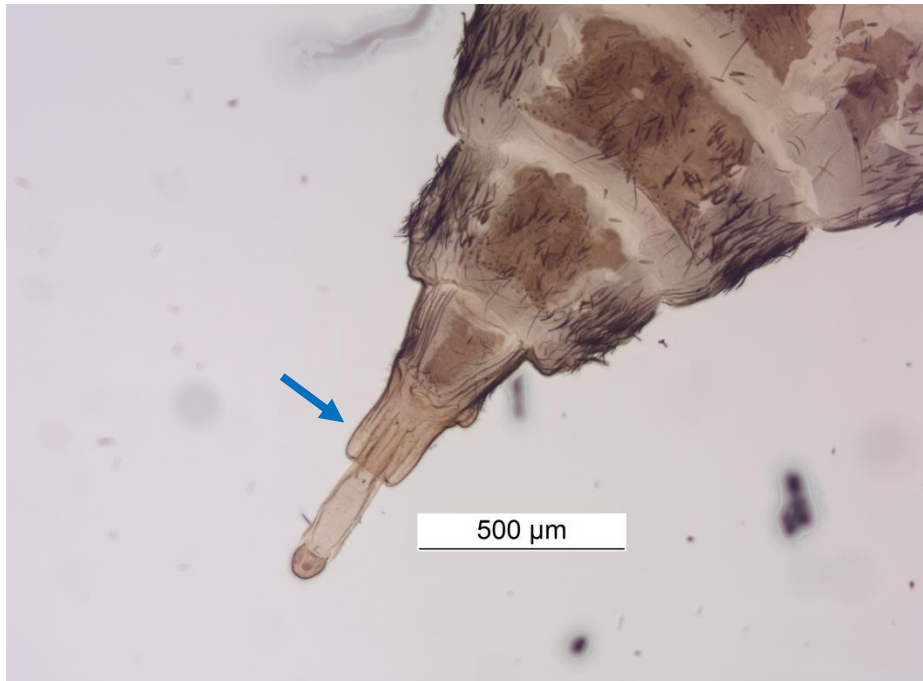


**Figure 11.** Pupae positioned at the base or crown of a wheat plant, no galls present. Photo: K.S. Pike, WSU (North Carolina Agricultural Extension Service)

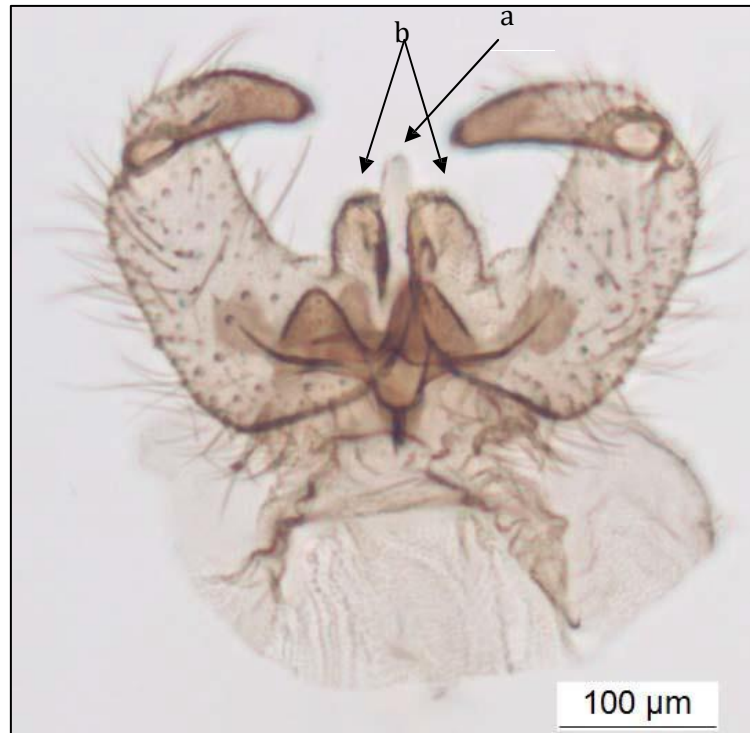




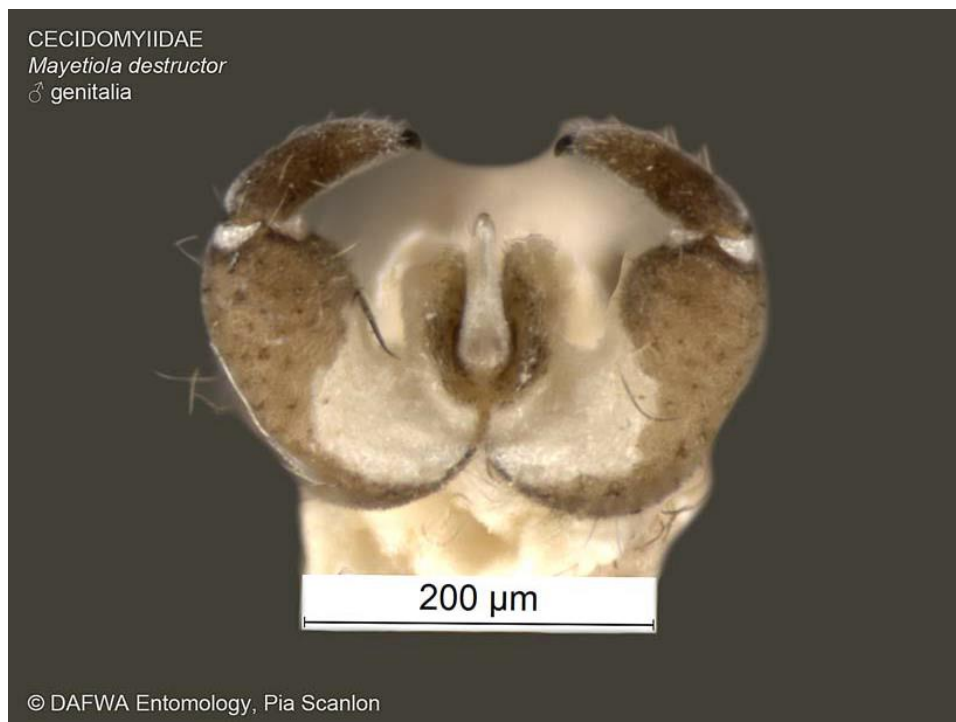
**Figure 12.** Female 7<sup>th</sup> tergite. Photo: S. Bauer ARS/USAD



**Figure 13.** Female 7<sup>th</sup> tergite flares out anteriorly. Photo: Dustin Severtson, Department of Agriculture and Food Western Australia



**Figure 14.** Hessian fly male terminalia cleared and mounted in Hoyer's medium showing (a) elongate gonostyli and (b) deeply separated hypoproctal lobes. Photo: Dustin Severtson, Department of Agriculture and Food Western Australia



**Figure 15.** Male terminalia of Hessian fly. Note the elongate gonostyli and deeply separated hypoproctal lobes as in Figure 14. Photo: Pia Scanlon, Department of Agriculture and Food Western Australia

## 4.3 Comparison with similar species

### 4.3.1 *Mayetiola hordei* Kieffer 1909

Synonyms: *Mayetiola mimeuri* (Mesnil, 1934)

Common name: Barley stem gall midge

Hosts: Barley

#### Comments

This species is not found on wheat (unlike the Hessian fly). Gagne *et al.* (1991) records the historic taxonomic confusion between the two species and their structural differences.

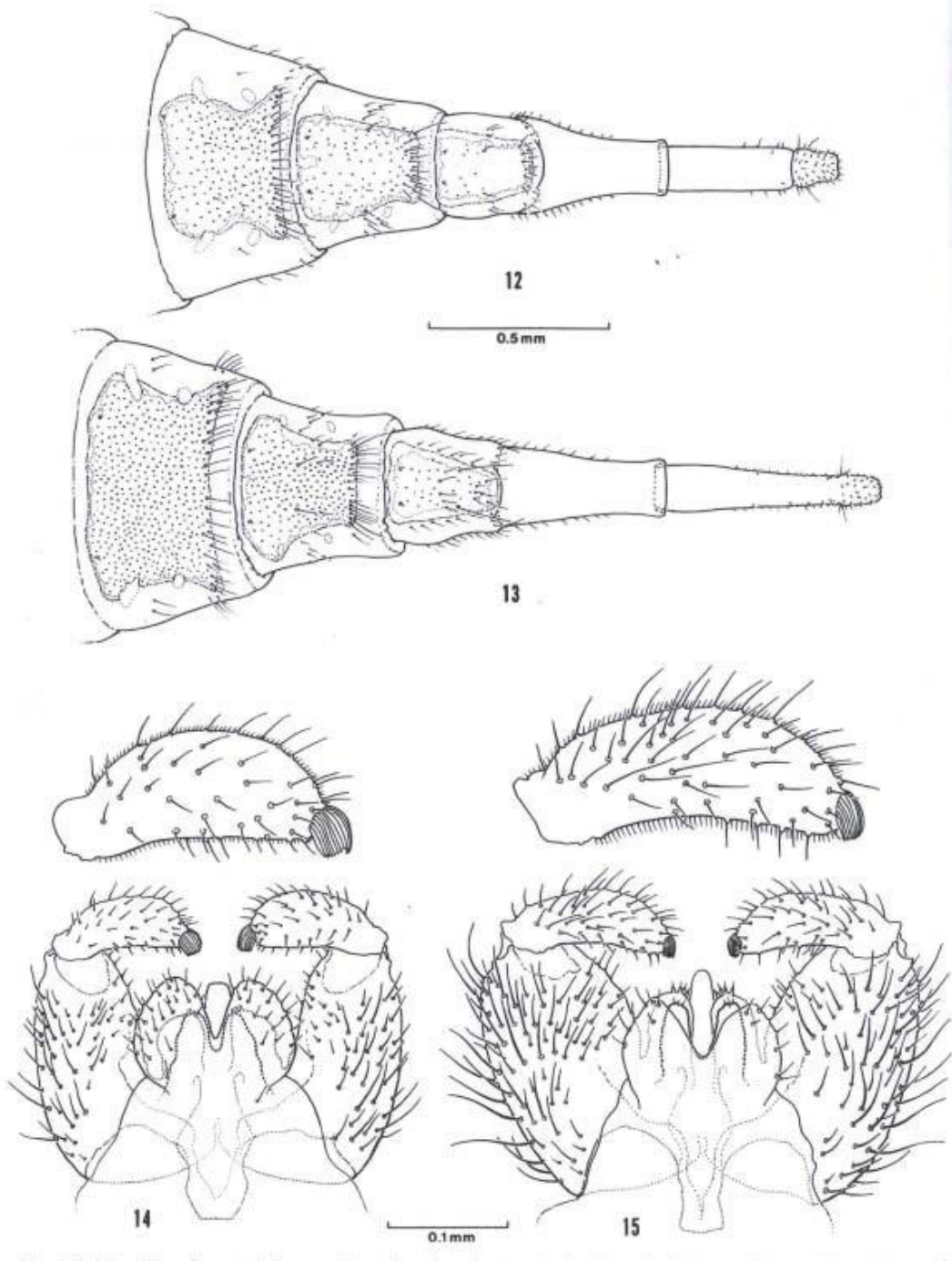
NB: *This species is also not found in Australia.*

External differences specific to *M. hordei*:

- Larvae induce pea-sized galls in host plant (Figure 16)
- Puparia almost entirely smooth
- Female genitalia has rectangular seventh tergite (Diagram 13 in Figure 17)
- Male genitalia have hypoproctal lobes which are triangular, and short gonostyli (Diagram 14 in Figure 17).



**Figure 16.** *M. hordei* gall in stem of barley plant.



**Figure 17.** Hessian fly and barley stem gall midge: Female postabdomens (dorsal) and male terminalia (dorsal) of two different species of *Mayetiola* (Diptera: Cecidomyiidae) in Morocco. (Source: Gagne R., Hatchett J., Lhaloui S., El Boussini M., 1991, *Annals of the Entomological Society of America*. 84:4, pp. 436-443).

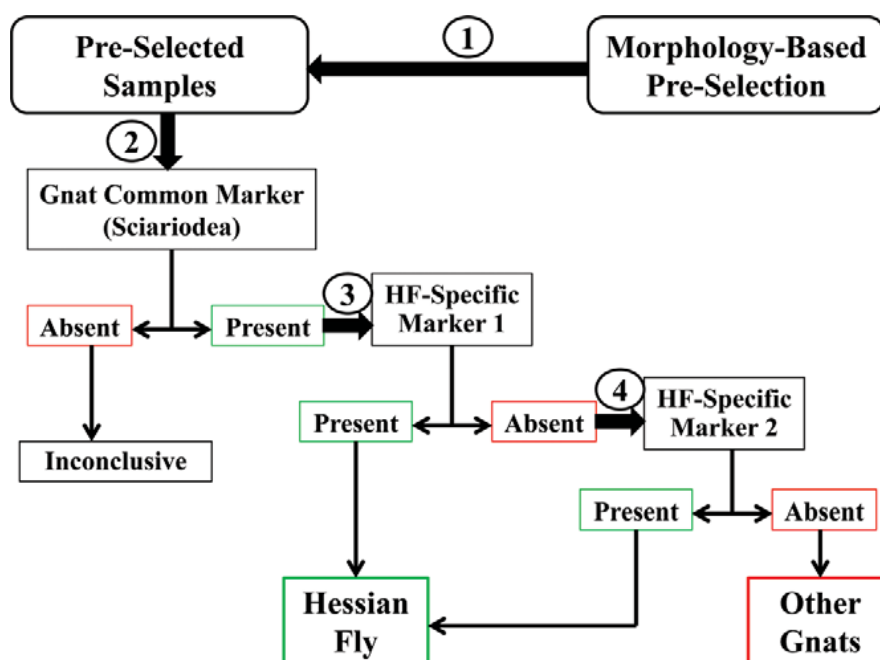
(12) female segments 6 to end of *M. hordei*; (13) same of *M. destructor*; (14) male terminalia with enlargement of left gonostylus of *M. hordei*; (15) same of *M. destructor*

## 4.4 Molecular identification

The PCR protocol for the identification of Hessian fly has been reproduced with permission from a published method in:

Chen, M.S., Wheeler, S., Davis, H., Jeff Whitworth, R., Knutson, A., Giles, K. L., et al. (2014). Molecular markers for species identification of Hessian fly males caught on sticky pheromone traps. *Journal of Economic Entomology*, 107(3), 1110-1117, doi:10.1603/ec13384.

The testing strategy recommended by Chen *et al* (2014):



**Figure 18.** Overall strategy to identify Hessian fly males on a sticky pheromone trap. The numbers within the circles above or beside a major arrow indicate four major steps in the identification process: 1) morphology-based preselection to exclude apparent non-midges, 2) Mycetophiloidea-common marker selection to exclude non-midge insects with similar morphology, 3) selection by Hessian fly-specific marker 1 to exclude non-Hessian fly midges, and 4) final selection by Hessian fly-specific marker 2 to reduce misidentification by specific marker 1 due to errors or gene sequence variation.

### 4.4.1 DNA Extraction.

Place individual insects into 1.5-ml Eppendorf tubes with 100  $\mu$ l STE buffer (10 mM Tris- HCl, 1 mM EDTA, pH 8.0, 0.1M NaCl) and homogenize with an electric microtube pestle. Incubate in boiling water for 5 min and centrifuge at 12,000 rpm for 5 min. Transfer the supernatant from each sample into a new 1.5 ml Eppendorf tube and add 250  $\mu$ l of -20°C ethanol. Invert the tube several times to mix the contents. Incubate at -20°C for 14-18 h and then centrifuge at full speed for 20 min at 4°C. Collect the DNA pellet and discard supernatant (aspirate the supernatant to avoid displacing the DNA pellet). Reconstitute the pellets with 30 $\mu$ l of ddH<sub>2</sub>O, vortex thoroughly, and store at -20°C for short-term storage or -80°C for long-term use.

#### 4.4.2 PCR amplification

Amplify according to the following programs:

For the common marker (CM, actin, AF017427) and Hessian-fly specific marker 2 (HFSM2, SSPG31-5, EV466578)

Steps	Temperature (°C)	Time (min)	Number of cycles
Denature	94	1	35x
Anneal	55	1	
Extension	72	2	

For the Hessian-fly specific marker 1 (HFSM1,MDP10, AEGA01028834)

Steps	Temperature (°C)	Time (min)	Number of cycles
Denature	94	1	45x
Anneal	55	1	
Extension	72	2	

A negative control without DNA template needs to be included for each PCR run. Optimisation may be needed due to various PCR mixes available and may differ between laboratories. In the first instance, test using the above PCR parameters. Marker specificity testing may require optimizing using different annealing temperatures (35, 40, 45, 50 and 55 °C) with the other PCR conditions remaining unchanged.

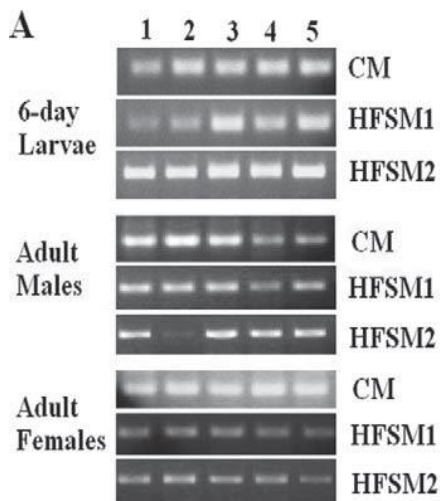
#### DNA primers

Marker	Primer sequence pair (5' - 3')	Targeted gene	Bp	Reference
Common	CACCAGCCATGTATGTTGCCAT AAACGGAGGATGGCATGTGGCA	<i>Actin A</i>	148	Chen <i>et al</i> 2014
Hessian fly-specific marker 1 (HFSM1)	TGCATTGCTACAACCTGAACGA CAACCGATTGTAGAACAG	<i>MDP10</i>	170	Chen <i>et al</i> 2013
Hessian fly-specific marker 2 (HFSM2)	AAAGTCATCATTTTGTAGCTTTGT TTATGCAGTGGTTGGAGTTGTT	<i>SSGP31-5</i>	369	Chen <i>et al</i> 2010



### 4.4.3 Interpretation

Bands will differ with the primer sets depending on developmental stages (Figure 19).



**Figure 19.** PCR amplification of DNA samples extracted from individual Hessian flies from different developmental stages with different primer sets. CM, HFSM1, and HFSM2 represent primers for the midge/gnat common marker, Hessian fly-specific marker 1, and Hessian fly-specific marker 2, respectively (Chen *et al* 2014).



## 5 CONTACTS FOR FURTHER INFORMATION

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Adjunct Professor, Department of Entomology, Kansas State University.

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Web: <http://www.k-state.edu/hessianfly/>

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The molecular procedure was reproduced by kind permission of MS Chen. Lucy Tran-Nguyen assisted with writing the molecular procedure in the required style.

The protocol was reviewed by Adrian Nicholas (NSW DPI) and Monica Kehoe (Department of Primary Industries and Regional Development, Western Australia)

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## 8 APPENDICES

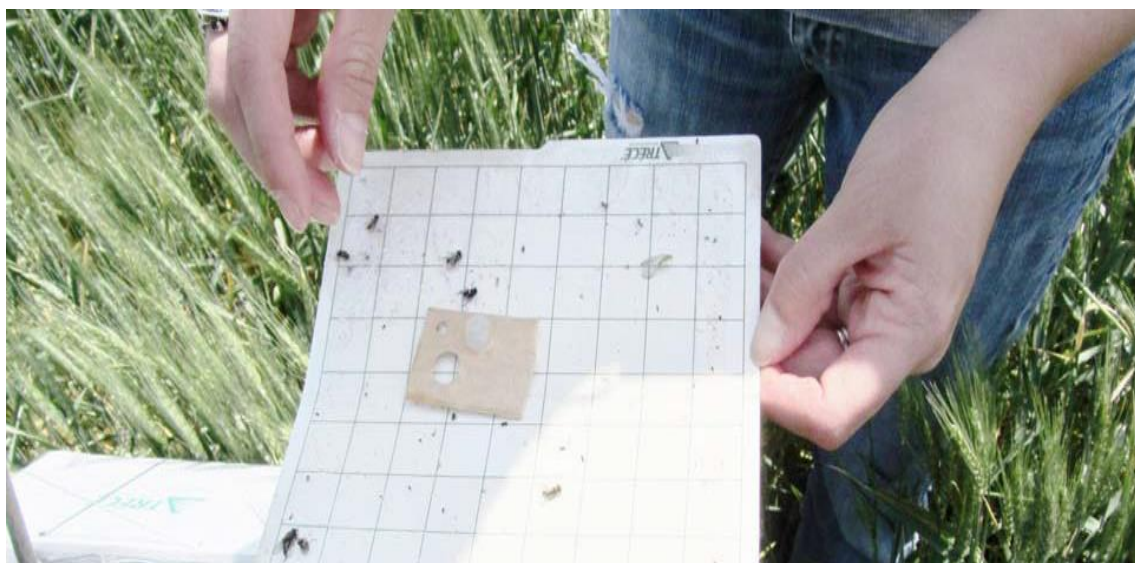
**Pheromone lures** for Hessian fly are available from [www.phero.net](http://www.phero.net). These are widely used in the U.S. where Hessian fly is present in wheat producing regions to investigate the fly's seasonal life cycle in localised conditions and warn growers of the risk of adult flies being present. The lures are placed in individual 'Delta' sticky traps (available from Trece® Incorporated) which are attached to a stake towards the ground up to approximately 20 cm above the soil for young crops or grasses. Vertical placement relative to crop height is important as Hessian flies are poor fliers and may not be able to reach the trap if placed too high (Figure 20). Traps may be placed higher in more advanced crops or grasses (i.e. heading) for detection of later generations of Hessian fly (Figure 21). Sticky traps may be placed unfolded in a transparent plastic (ziplock) bag for transport and later inspection (Figure 22).



**Figure 20.** Delta sticky trap with Hessian fly pheromones.



**Figure 21.** Delta sticky trap with Hessian fly pheromone placed higher in an advanced wheat crop.



**Figure 22.** Delta trap showing flies and pheromone.