Diagnostics[1]
Diagnostic

Popillia japonica

Specific scope

This standard describes a diagnostic protocol for *Popillia japonica*.

Specific approval and amendment

Approved in 2006-09.

Introduction

Popillia japonica originates from Asia where it is native in northern China, Japan and the Far East of Russia. It was introduced into North America and has become a more serious pest in the USA than in its area of origin. Further information can be found in the EPPO data sheet on *P. japonica* (EPPO/CABI, 1997).

Identity

Name: *Popillia japonica* Newman
Taxonomic position: Coleoptera Scarabaeoidea Rutelidae
[Nomenclature and taxonomy suggested by Fauna Europaea are used as the reference]
Common name: Japanese beetle
EPPO computer code: POPIJA
Phytosanitary categorization: A1 pest recommended for regulation; EU annex: IA1.

Detection

Adults can be detected by visual examination of green parts of plants and larvae by visual examination of roots in soil. Traps containing food-type lures and/or sex attractants have been widely used in the USA to monitor populations and could be useful in warehouses with imported commodities.

Popilia japonica is a polyphagous species, feeding on at least 295 species of plants. *Acer*, *Malus*, *Prunus*, *Rosa*, *Rubus*, *Vitis* and *Zea mays* are the main host of concern in the EPPO area.

Symptoms caused by adults of *P. japonica* are easily recognizable (defoliation). The beetle skeletonizes the leaves by chewing out the tissue between the veins and leaving a vein skeleton. Leaves may turn brown and fall. On flower petals, the beetle consume large and irregularly shaped parts. Infestation of maize results in an increase in the number of embryonic and malformed kernels. The larvae simply cause feeding damage to the roots of host plants, and the symptoms caused are not all specific.

Identification

Morphological identification with a binocular microscope is the recommended diagnostic method.

For keys to Coleoptera families see Downie & Arnett (1996) (nearctic), Delvare & Aberlenc (1989) (afrotropical), Baraud (1992) (west palearctic).

For a key of Scarabaeoidea families and the *Popillia* genus see Appendix 1.

They are some limitations to this method as the genus *Popillia* is composed of more than 300 species, most of which are from Africa and Asia. The probability of misidentifying specimens coming from North America is very low but it does exist for individuals detected in imported consignments from Africa or Asia, for example, for *Popillia lewissi* and *Popillia* sp. nr. *taiwana* (http://micronesianinsects.com/IIR/2004.02.htm).

For a check-list of oriental *Popillia* see http://www3.famille.ne.jp/~kazuo/popi.html.

There are no exhaustive keys that will identify these species. Sabatinelli (1993, 1994) gives notes on several Asian species. Paulian (1958) gives keys for species from South-Eastern Asia.

[1]The figures in this standard marked 'Web Fig.' are published on the EPPO Website at: www.eppo.org.

Description of *P. japonica* stages (after USDA-APHIS-PPQ-CPHST-PERAL/NCSU):

Eggs: Newly deposited eggs may be quite variable in size and shape: spheroids with a diameter of 1.5 mm, ellipsoids 1.5 mm long by 1.0 mm wide, or nearly cylindrical. Colour may range from translucent to creamy white and the external surface is marked with hexagonal areas. The eggs enlarge to nearly double their initial size and become more spherical as the embryo develops within the chorion.

Larvae (Web Fig. 1): Upon eclosion, the first larval instar is completely white, 1.5 mm in length with biting mouthparts, three pair of thoracic legs and 10 abdominal segments. The larval body is typically found in a C shape, which is referred to as scarabaeiform. Within a few hours after eclosion, the head and spiracles of the larvae sclerotize to a light yellow brown colour. After initiation of feeding, a greyish to black colour may appear in the posterior region of the abdomen. The body of the larvae is covered with a scattering of long brown hairs and interspersed short blunt spines. The ventral side of the tenth abdominal segment bears two medial rows of six-seven spines in a characteristic V shape (Web Fig. 2). The V shape is unique to *P. japonica* and may be used to distinguish it from other species of scarab larvae (see Sim, 1934; Klausnitzer, 1978). The first larval instar is distinguishable from the subsequent instars by the presence of a rigidly pointed process on each side of the metathoracic scutellum and lack of a concave respiratory plate surrounding a bulla with a curved spiracle slit. The second and third instar larvae can be separated by head capsule size, the head capsule size of the second larval instar being 1.9 mm wide and 1.2 mm long and the third instar being 3.1 mm wide and 2.1 mm long.

Prepupa: The larva is mature, but feeding ceases, excrement is evacuated and activity is reduced as internal changes occur.

Pupae: The pupae are 14 mm in length and 7 mm wide on average and exarate in form. Pupae resemble the adult, but wings, legs and antennae are held close to the body and functionless. The colour changes from a cream colour to tan and eventually the metallic green observed in the adult. Only male have a three-lobed eruption covering the developing genitalia on the posterior ventral abdominal segments so can be distinguished from females.

Adult (Web Fig. 3): The adult beetle is brightly colored metallic green and coppery bronze, oval in shape, and varies in size from 8 to 11 mm in length and 5 to 7 mm wide. The female is typically larger than the male. Along each lateral side of the elytra there are five tufts of white hair present and two dorsal spots of white hair on the last abdominal segment. Male and female beetles can be differentiated from each other by the shape of the tibia and tarsus on the foreleg (Web Fig. 4). The male tibial spur is more sharply pointed and the tarsi are shorter and stouter than those of the female.

Examination of the male genitalia after preparation can supplement the visual identification of the habitus (Kim, 1995; Ku *et al*., 1999). For preparation see Appendix 2. Preparation should be compared with Web Fig. 5.

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM 7/77 (1) Documentation and reporting on a diagnosis.

Acknowledgements

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Glossary

Clypeus: anterior sclerite of the head below frons (face) and above the labrum

Pronotum: the upper or dorsal surface of the first thoracic segment (Prothorax)

Spiracles: a pore, hole or aperture in the integument which serves as an adaptation to permit gas exchange between the body and the environment

Sternite: the ventral part of a sclerotized, ring-like body segment which is separated by membrane from sclerotized lateral or dorsal elements of the segment

Tergite: a dorsal sclerite which is part of a body segment or sclerotized ring of a generalized body segment.

Appendix 1

Seventeen families of Scarabaeoidea are present in Europe (see *Fauna Europaea* website). The parallel use of the keys proposed by Baraud (1992) and Arnett *et al*. (2002) allows the identification of the *Popillia* genus. Jameson and Ratcliffe (2000) present a useful key on their web site.

They are clear differences in approach in the keys leading to the family level between Europe and, for example, the USA. Both the European and USA keys are valid for *P. japonica*, although this does not prejudge their effectiveness for identification of other species of the genus.

Superfamily Scarabaeoidea

Key for adults of the European families (after Baraud, 1992):

1

• Penultimate abdominal tergite forms, with corresponding sternite, an incomplete ring, not very sclerified at the junction. Spiracles of abdominal segments located on the membranous linking sternites and tergites. Elytras often cover pygidium.	**Laparosticti** (Scarabaeidae, Aphodiidae, Trogidae, Ochodaeidae, Geotrupidae, Hybosoridae, Orphnidae)
• Penultimate abdominal tergite forms, with corresponding sternite, a complete ring (Web Fig. 6), well sclerified. Abdominal piracles of at least last segments located in the upper part of sternites (Web Fig. 6). Elytras do not cover pygidium.	**Pleurosticti 2**

2

• Clypeus not indented at its base, antennal insertion not visible from above.	**3**
• Clypeus deeply indented at its base, antennal insertion visible from above.	**Glaphyridae et Cetoniidae**

3

• Claws of the median and especially posterior tarsi unequal, shortest always whole; separately mobile.	**Rutelidae**
• Claws of the median and posterior tarsi equal and not mobile; seldom unequal but then the two claws are split; posterior tarsi sometimes finished by only one claw.	**Dynastidae, Melolonthidae and Euchiridae**

Family Rutelidae (after Arnett *et al*., 2002)

Arnett *et al*. (2002) consider *Rutelinae* as a subfamily but in recent taxonomic work most consider it as a family.

1

• lateral margin of elytra with membranous border (Web Fig. 7) (Anomalini).	**2**
• lateral margin of elytra lacking membranous border.	**Rutelini**

2

• Protibial spur absent; lacinia reduced, with 2 or fewer teeth.	***Leptophila***
• Protibial spur present, sometimes small in the female; lacinia not reduced, with more than two teeth (Web Fig. 8).	

3

• Base of pronotum tri-emarginate (Web Fig. 9); pygidium and abdominal sternites at sides with patches of dense, white setae (Web Fig. 6).	***Popillia***
• Base of pronotum rounded posteriorly; pygidium and abdominal sternites at sides without patches of dense, white setae.	**other Rutelidae**

Appendix 2

Preparation of male genitalia for observation under a binocular microscope

Extract the final third of the abdomen and macerate by warming to around 80°C in a 10% potassium solution for 10–20 min. The body contents will be dissolved.

During this time, by dissecting eliminate all the sclerite composing the abdomen and preserve only the genitalia.

Rinse the genitalia in cold distilled water.

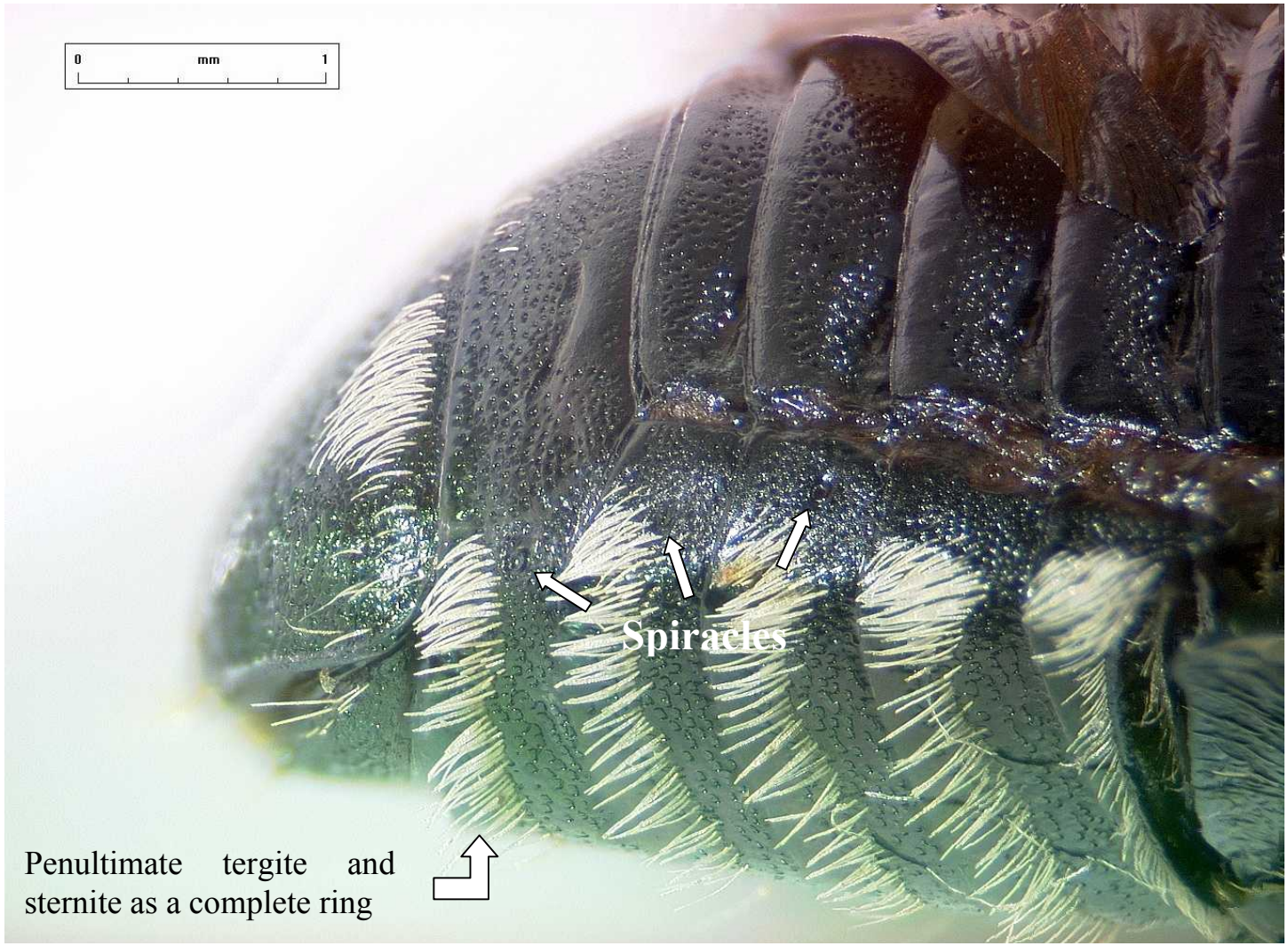
Observation of the genitalia will be realized in 70% alcohol or glycerine. After dissection the genitalia to be enclosed in a drop of glycerine in a glass or plastic microvial which will be pinned below the specimen.

Web Fig.3: Habitus (male).
(photo Germain LNPV)

Web Fig.4: Protibial aspect, male pointed, female rounded
(photos Germain LNPV)

Lateral view | Dorsal view | Ventral view

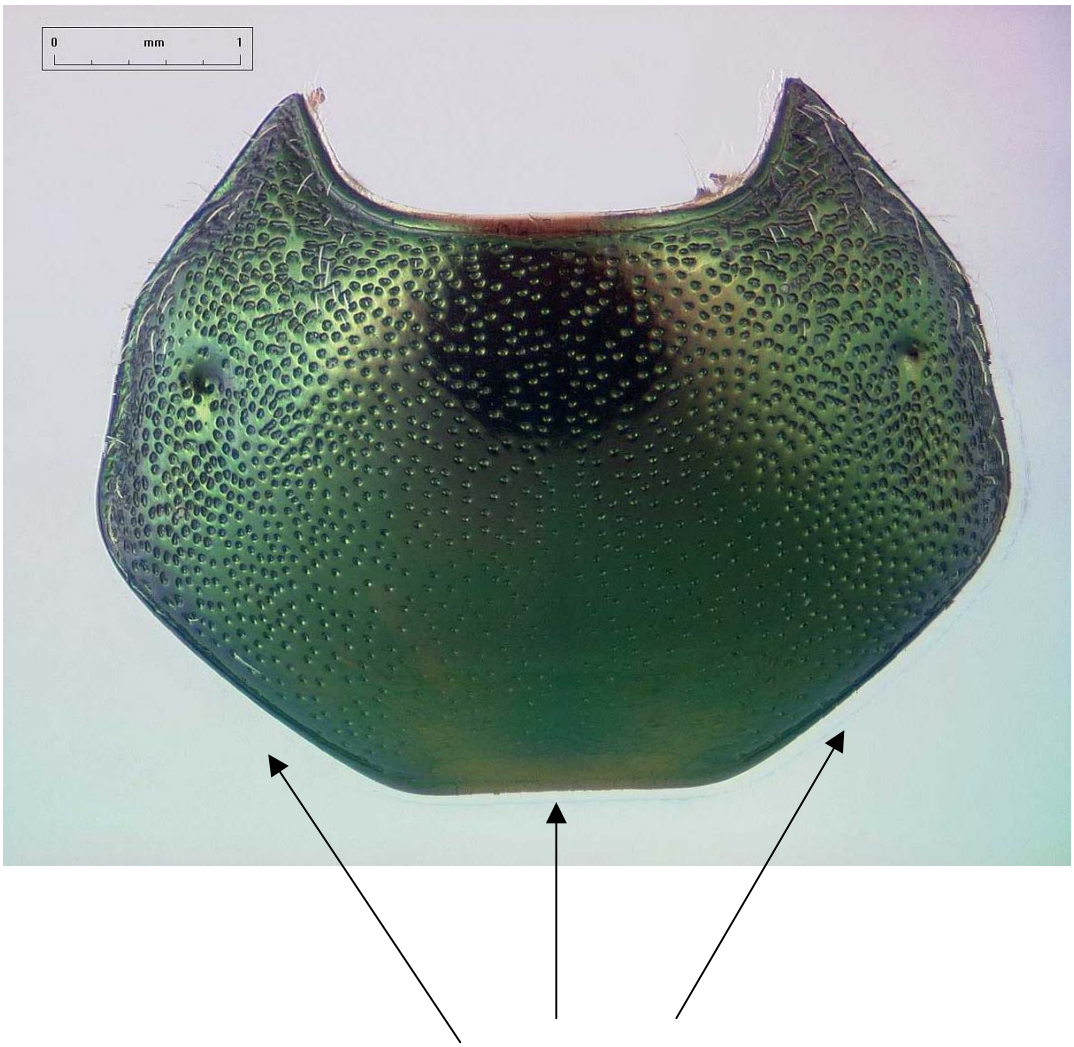
Web Fig 5: Male aedeagus
(photos Germain LNPV)



Web Fig. 6 : Abdomen, lateral view
(photo Germain LNPV)

Web Fig. 7 : Elytra, membranous border of lateral margin
(photos Germain LNPV)

Web Fig. 8 : Lacinia with more than 2 teeth
(photo Germain LNPV)



Web Fig.9: Pronotum aspect
(photo Germain LNPV)