Bulletin OEPP/EPPO Bulletin (2016) **46** (3), 543–555 ISSN 0250-8052. DOI: 10.1111/epp.12345

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 9/21(1)

National regulatory control systems
Systèmes de lutte nationaux réglementaires

PM 9/21(1) *Popillia japonica:* procedures for official control

Scope

This Standard describes procedures for official control with the aim of detecting, containing and eradicating *Popillia japonica*. NPPOs may draw on this guidance when developing national contingency plans for outbreaks of *Popillia japonica.*

Approval and amendment

First approved in 2016-09

1. Introduction

Popillia japonica Newman (EPPO Code: POPIJA) (Coleoptera: Rutelidae), commonly known as the Japanese beetle, is a highly polyphagous beetle and an EPPO A2 pest (Potter & Held, 2002; EPPO, 2006). *Popillia japonica* is listed in Annex IAII of the Directive 2000/29/EC, so any detection on consignments entering European Union (EU) Member States would be subject to statutory action. Native to Japan and the far eastern Russian island of Kuril, *P. japonica* has become an established pest in North America, the Azores and more recently in Europe in the north of Italy (EPPO, 2014a).

Popillia japonica was first recorded in the United States in 1916 from a nursery near Riverton in New Jersey (Fleming, 1972; Petty *et al.*, 2015). The species is now widespread throughout the eastern States with the exception of Florida (Fleming, 1972; Oklahoma State University, 2010; Oregon Department of Agriculture 2010; University of Florida, 2014). In Canada, the pest is known to occur in the southern parts of Ontario and Quebec. In Nova Scotia, in the 1940s, there was an outbreak of the pest but this was eradicated in the early 1950s (EPPO, 2003). However, the species has been recorded since, in 2001, from Cornwallis Park (Halifax) (EPPO, 2003). Additional reports of a small number of individuals (one or two adults) have been reported from Newfoundland, though it is believed these were transient rather than representing an established population (Defra, 2015). Reports of the species being present in the Republic of Korea are likely to be erroneous, considering a comprehensive examination of the genus and a comprehensive survey which failed to find any specimens. Earlier records are likely to be misidentifications and are most probably *Popillia quadriguttata* (F.) (EPPO, 2000). Records of the species in China are regarded as invalid or unreliable records.

Within the EPPO region, *P. japonica* was first identified from the island of Terceira in the Azores (PT) in the early 1970s, and has since been recorded from the islands of Faial, Flores, Pico, São Jorge, Corvo and on the western part of São Miguel (Simões & Martins, 1985; Martins & Simões, 1986; Vieira, 2008). In 2014, *P. japonica* was recorded for the first time on the European mainland when an outbreak was reported within the Ticino Valley Natural Park, Italy (EPPO, 2014; Pavesi, 2014). Approximately 180 adults were collected along a 2-km stretch near Turbigo (Milan Province) (Pavesi, 2014). Although the pathway for entry is unknown, it has been noted that two airports (one civil and one military) are located close to the infested area. In Russia, *P. japonica* has been recorded from the Kuril Islands, though only a sporadic occurrence was reported where the pest was observed during a survey in 2011 (EPPO, 2014a).

Life cycle

Eggs are usually laid in moist grassland in the summer. Larvae feed on roots throughout the autumn and spring, overwintering 15–20 cm below ground, and then pupate nearer the surface, emerging as adults to feed on leaves of a wide range of host plants (see the sections on host plants and Table 1).

Throughout most of its native and exotic range, *P. japonica* is univoltine with three larval instars. The adult

Table 1. Potential preferred hosts of adult *Popillia japonica* (species are mentioned when they are particularly important)

Key genera	Including species
Acer	
Centaurea	*Centaurea phrygia*
Corylus	*Corylus avellana*
Filipendula	*Filipendula ulmaria*
Glycine	*Glycine max*
Hibiscus	
Humulus	*Humulus lupulus*
Malus	
Oenothera	*Oenothera biennis*
Parthenocissus	
Prunus	*Prunus avium, P. persica*
Rosa	
Rubus	
Rumex	
Salix	
Tilia	
Ulmus	
Urtica	*Urtica dioica*
Vitis	
Zea	*Zea mays*

life span is 4–6 weeks (Potter & Held, 2002). In cooler years in New England (US), some of the population can take 2 years to complete a generation (Potter & Held, 2002). In Italy, the life cycle is completed in 1 year, with adults active between June and August and peak activity in mid July (Regione Piemonte, 2015). Adults may still be found in September, and in 2015 there was one finding in October (Regione Piemonte, 2015). In the Azores, the flight period of adult beetles can extend from late May through to early November – the peak number of adults caught in traps was in the second half of July and the first half of August (Martins & Simões, 1988; Vieira, 2008).

Feeding and mating begins soon after the adults emerge from the soil in late June (Potter & Held, 2002). Females mate repeatedly and return to the soil after each mating to lay a total of between 40 and 60 eggs (Potter & Held, 2002). Eggs may be laid singly or in small clusters. Females may form a burrow in the upper 10 cm of soil to deposit their eggs. Eggs hatch after approximately 10–14 days and the first and second instars last approximately 2–3 weeks and 3–4 weeks, respectively (Potter & Held, 2002). The larvae feed on grass roots for the remainder of the summer months (upper 5 cm of the soil; Martins & Simões, 1988) burrowing deeper into the soil (>15 cm) to overwinter. The following spring, the larvae migrate towards the surface and feed on grass roots for several weeks. The final instar forms an earthen cell in which to pupate and after 7–17 days emerges as an adult when the local conditions are favourable. Martins & Simões (1988) detail that emergence begins by the end of May in the Azores. (See Appendix 1 for a detailed description of the stages of *P. japonica*.)

Signs and symptoms

Symptoms indicative of adult beetles include feeding holes in host plants extending to skeletonization of leaves when population numbers are high. Often the mid-vein of leaves is left intact. Severely damaged leaves soon turn brown. They may drop or remain attached. On some plants with thin leaves and fine venation, and on petals of flowers, the beetles consume irregularly shaped sections in the same manner as many Lepidoptera (CABI, 2015). The adults are gregarious and have been reported from the USA as usually beginning to feed on foliage at the top of a plant and working downward (Fleming, 1972). Evidence of the presence of *P. japonica* larvae in the soil can be seen in the discolouration of grass in patches which gradually enlarge over time. Severe infestations can result in the death of the turf (Vail *et al.*, 1999). More details and pictures are given in Appendix 1.

Natural spread

Fleming (1972) reports that most flights of adult beetles are for short distances. In the USA, adults move between plants frequently and marked beetles have been found 2.75 miles (3.2 km) away from their original point of capture (Fleming, 1972). The Plant Protection Service of Lombardy (Milan, IT) (2016) reported that the adults can fly for a distance of about 500 m, which is similar to studies from the USA where a mark–release–recapture experiment recorded flight distances of up to 500 m during the day, though sometimes up to 700 m (Hamilton, 2003). Holmes & Barrett (1997) recorded the longest distance of 400 m following a mark and recapture experiment. Flight activity is reported to be greatest on clear days and when the temperature is between 29°C and 35°C, relative humidity >60% and wind is <20 km h^{-1} (Potter & Held, 2002). Much longer distances have been reported; adult beetles are capable of flying 5 miles (8 km) over water in sustained flights (Fleming, 1972). In Italy, in 2016, the spread of adults has increased as a result of population increase (Mariangela Ciampitti, Regione Lombardia, pers. comm., 2016).

Outward spread of the population from the point of introduction has been reported in the USA at a rate of 10–15 miles (16–24 km) per year (Smith & Hadley, 1926). Other estimates of outward movement from an infestation varied from 2–10 miles (3.2–24 km) per year [with an average of 5 miles (8 km) per year] (Fox, 1932). In the Azores, the original spread was slow, but between 1984 and 1985 the infested area increased by a minimum of 2 km each year (Martins *et al.*, 1988).

Host plants for adults

This pest is a highly polyphagous species and the adults can be found feeding on a wide range of trees, shrubs, wild plants and crops (Fleming, 1972; Vieira, 2008). In the introduced range in the USA, the host range of adult beetles is extensive, with over 300 host species recorded from 79 plant families (Potter & Held, 2002; VirginiaTech, 2009). In Japan, however, the host range is considerably more restricted. In the Azores, the beetle feeds on a wide range of hosts including *Medicago sativa* (alfalfa), *Acer* spp. (maples), *Phaseolus vulgaris* (pea), *Populus* spp. (poplar), *Asparagus officinalis* (asparagus), *Glycine max* (soybean), *Malus* spp. (apples), *Prunus* spp. (stone fruit including plums, peaches, etc.), *Rosa* spp. (roses), *Rubus* spp. (blackberry, raspberry), *Tilia* spp. (lime trees), *Quercus* spp. (oaks), *Ulmus procera* (English elm), *Vitis* spp. (grapes) and *Zea mays* (maize) (Vieira, 2008). At the Ticino Valley outbreak site in Italy, *P. japonica* was observed on wild plants (*Rubus*, *Ulmus*, *Urtica, Rosa*, *Populus* and *Parthenocissus*) and crops of soybean (*Glycine max*) (EPPO, 2014a).

From early experience of the pest in Mainland Europe (Italy) it appears that the genera and species listed in Table 1 may be preferred hosts for adults and could be included as such in survey plans. This list is not intended to be a comprehensive host list and the pest may show distinct preferences within a genus or even a species. For a comprehensive list of host plants from the USA see Fleming (1972).

In the Ticino Valley outbreak in 2015, the most significant damage by adults was on fruit trees (*Prunus avium*, *Prunus persica*). The regional plant protection service expects to see damage on more crops if populations increase.

Host plants for larvae

Food plants for larvae are less well known as larvae live and develop below ground (Potter & Held, 2002) so there is considerable uncertainty about the potential number of plant species on which this pest can complete its life cycle. The larval food source is mostly determined by which plants are growing in the area where the female beetle oviposits (Fleming, 1972). *Popillia japonica* can cause significant damage to nurseries, seedbeds, orchards, field crops, landscape plants, turf and garden plants (Oregon Department of Agriculture, 2010). In the 2015 Ticino outbreak the most visible damage was indirect and caused by animals such as wild boar foraging for larvae in grassland. The main species within the grassland were of the genera *Festuca*, *Poa* and *Lolium*.

2. Contingency planning

All EPPO countries in which conditions are suitable for the establishment of *P. japonica* are encouraged to develop and test a contingency plan for outbreaks of the pest. NPPOs are advised to draw on the guidance in this Standard and in EPPO Standards PM 9/10 (1) *Generic elements for contingency plans* EPPO (2009) and PM 9/18 (1) *Decision-support scheme for prioritizing action during outbreaks* EPPO (2014b), and on recent experience with the pest in the EPPO region and elsewhere.

3. Surveillance

Surveillance in an area where *P. japonica* is not known to occur

In an area believed to be free from *P. japonica,* surveillance is based on:
- carrying out a detection survey and
- raising awareness.

Detection surveys

The detection survey should be based on visual inspection for adults and larvae, and on trapping, with a focus on high-risk sites (see below), using a lure based on a combination of sex pheromones and floral attractants (which normally lasts for one season). Because this mixed lure may attract both male and female beetles into a previously pest-free area, possibly from up to 1 km away, it should not be used in buffer zones surrounding known infested areas (see below).

In order to verify and ensure that an area is free from *P. japonica*, periodic surveys should be carried out (according to ISPM 4 *Requirements for the establishment of pest free areas* and ISPM 6 *Guidelines for surveillance*: FAO, 1996, 1997, respectively). These surveys should be conducted annually in countries known to have suitable conditions for establishment.

Detection surveys need to locate adult beetles above ground and/or larvae below ground (Potter & Held, 2002) (see Appendices 2 and 3 respectively). When designing a survey strategy, the following aspects should be considered: key entry points, transportation networks from these entry points and suitable habitats for oviposition and larval development - particularly areas with abundant moist grassland.

Survey criteria to detect the presence and confirm the absence of *P. japonica* should be based on factors associated with (1) transport and (2) the biology of the pest.

(1) Transport: adult beetles have been reported as entering areas as hitchhikers on non-host commodities and vehicles (Hamilton *et al.*, 2007; DEFRA, 2015). High-risk areas for points of entry include: major entry points such as airports (civil and military), ferry docks, bus stations, railway stations and distribution centres (Lombardy, 2016; Hamilton *et al.*, 2007). It is interesting to note that in the Azores, initial infestations of *P. japonica* were restricted to fields surrounding airports and ferry ports (Vieira, 2008). In Scotland, a *Popillia* species was intercepted with computer parts from Taiwan (DEFRA, 2015). Surveys should target those pathways with the highest likelihood of *P. japonica* being present. The main pathways include,

for adult beetles, hitchhiking on commodities and vehicles, and, for larvae, soil, either on its own or with plants for planting (DEFRA, 2015).

(2) The biology of the pest: the key factors to take into account from the biology of the pest are that females have a preference for certain types of oviposition sites (e.g. moist grassland), and that the adult beetles are more likely to be seen on certain favoured host plants (see Table 1).

In the areas where the pest is not known to occur, monitoring of high-risk sites (as detailed below) should be carried out using traps:

- airports, ferry docks, bus stations and railway stations
- distribution centres,
- sites with abundant suitable habitat in the vicinity of outbreak areas (but outside the buffer zone).

Traps with a combined pheromone and floral attractant lure should be put up at the end of May, checked once a month during the summer (the frequency can be reduced at low-risk sites) and collected in September. Any beetles found should be examined and, if the distinctive features of *P japonica* are present, those individuals should be sent to the laboratory for confirmation.

Traps can attract beetles from distances of several hundred metres (Hamilton *et al.*, 2007). For other species there is evidence that placing pheromone traps too close together can cause confusion and reduce their effectiveness for monitoring purposes. In the absence of specific evidence on this point for *P. japonica*, it is recommended that traps for detection surveys should not be placed closer than 200 m apart.

At high-risk sites traps should be placed near specific potential sources of introduction, such as unloading bays. More details of how to use traps are provided in Appendix 4.

Public awareness

Adult beetles can be distinguished from other species by well-informed stakeholders such as growers and amateur naturalists. Raising awareness among these groups may help to ensure early detection of an outbreak. For example, the outbreak in the Ticino Valley was originally discovered as a result of a report from an amateur naturalist.

Information campaigns targeted at the general public are not recommended for this pest away from outbreak areas because they are likely to produce a large number of false reports.

From experience in the USA, the development and initiation of an outreach and education campaign can be critical to the success of eradication programmes (UDAF, 2015). Awareness activities should especially target those trading in plants and plant products, agencies and stakeholders working in high-risk areas (e.g. parks, nurseries, golf courses, entry and exit points). This is very important for early detection and reduced spread of *P. japonica*. Awareness raising can be achieved, for example, via the internet and mobile apps, and through workshops involving land managers, growers, gardeners, entomologists, etc.

Fact sheets should be provided to aid the detection and identification of *P. japonica*. Appendix 1 provides text and pictures which may be used by NPPOs in producing such fact sheets.

Obligation to report findings

An official requirement to report findings and communication systems should be in place so that each finding or suspicion of *P. japonica* is immediately reported to the NPPO.

Detection in a new area

If *P. japonica* is confirmed in an area where the pest was previously not known to occur, immediate action should be taken to confirm whether this is the result of a locally established population or just detection of one or more transient individuals. This should be determined initially by increasing the number of traps in the locality and the frequency with which the traps are checked, then, depending on the time of year, by visual inspection of host plants and inspection of grassland soil for larvae. As reported in PM 9/15 (1) *Anoplophora glabripennis: procedures for official control* (EPPO, 2013), from experience with other pests it should not be assumed that the initial finding is a centre of infestation. This is especially the case if the pest is detected by trapping.

Based on all the information available, a decision should then be taken and recorded as to whether there is likely to be a locally established population. Where this is the case, the NPPO should demarcate a zone comprising the infested or probable infested zone and a buffer zone of at least 3 km in radius. The boundaries of the buffer zone should be established taking account of the following factors:

- the estimated population level of the pest
- suppression measures being taken
- the distribution of suitable habitat within the area
- natural barriers to the spread of the pest
- the intensity of surveillance within the buffer zone and outside the buffer zone.

Within the demarcated zone the following measures should be implemented:

- a delimiting survey
- monitoring surveys
- eradication measures
- containment measures.

These are described below in turn.

A report of the finding should be provided as soon as possible to EPPO by the NPPO. Immediate consideration should be given to seeking direct guidance from experts in other countries who have experience of dealing with outbreaks of the pest. The EPPO Secretariat may be able to provide contact details.

Delimiting survey

The purpose of a delimiting survey is to determine the geographical limits of the infested or probable infested zone (or areas) and a buffer zone and ensure they are correctly demarcated. The survey should start near the location of the first findings and work outwards. In order to identify the infested area, densities of traps should be increased in the area surrounding the finding (see Appendix 4). If the presence of *P. japonica* is confirmed at new sites, a larger area than just the infested area may be designated as the probable infested area. The buffer zone should be reviewed and changed accordingly. The declaration of larger areas as probably infested enables trapping to be undertaken over a wider area, which increases the chance of further detection and the likely effectiveness of trapping when used as a method of control.

The survey should focus on habitats classified as high risk for oviposition and on favoured host plants for adult beetles. Within the buffer zone, traps using a combination of sex pheromone and floral attractant should not be used because the combined lure may attract beetles of both sexes from the infested area into a previously pest-free area. Inspections within the buffer zone should therefore be based on visual inspection for beetles and associated damage on favoured host plants during the summer, and examination of soil samples for larvae during the autumn and spring (see Appendices 3 and 4).

Traps may be used when spread of the pest into the buffer zone is suspected, but they should be baited with a sex pheromone lure only (without the floral attractant which may attract females) and only exposed for short periods (a few hours) before being removed. This is to reduce the risk of attracting adults into previously pest-free areas.

Larvae can be detected by visual examination of soil and roots. Swards heavily damaged by *P. japonica* larvae can feel spongy and are easily separated from the soil substrate due to the larvae feeding on the roots. Microscopic identification may be required to distinguish *P. japonica* larvae from closely related species. A distinctive morphological characteristic of *P. japonica* larvae is a series of spines (*raster*) located on the last segment of the abdomen, 6–7 in number, ventral to the anal opening and arranged in a 'V'. These may be seen with a hand lens. If they are not present, the larva belongs to a species other than *P. japonica*. More details on identification are provided in PM 7/74(1) *Popillia japonica* and Appendix 1.

Molecular identification methods are available for distinguishing larvae from native species, including PCR testing.

Monitoring surveys within the infested area to assess the effectiveness of measures

Monitoring surveys, based on trapping and visual inspection for adult beetles and inspection of soil samples for larvae, should be conducted within the infested area to determine the distribution and population levels of the pest. This information should be used to target control measures and monitor their effectiveness. To identify the period when the adults begin to emerge, traps should be placed at sites that yielded the most captures in previous years.

4. Eradication measures

There have been examples, mainly from the USA, of both successful and unsuccessful eradication strategies in newly infested areas (Clair & Kramer, 1989; Vittum *et al.*, 1999; Pluess *et al.*, 2012). A programme for pest eradication may be developed as (1) an emergency measure to prevent establishment and/or spread of a pest following its recent entry or (2) a measure to eliminate an established pest (FAO, 1998). Following the official confirmation of *P. japonica* in an area, a combination of measures should be taken to reduce the population with a view to eradication, if possible, and to prevent spread. Measures to reduce the population will also assist with containment by reducing the risk of passive and active spread. There is limited experience of the application of such measures in the EPPO region at the time of writing. It is expected that further information on the effectiveness of different measures will become available and will be incorporated into future revisions of this Standard.

Trapping as a control measure

Traps containing a combination of floral attractant and synthetic pheromone lures can be used to suppress populations as well as to monitor the spread and development of *P. japonica* (Switzer *et al.*, 2009). The use of mass trapping is likely to reduce isolated populations so will be an important part of most eradication campaigns. The use of traps as a form of suppression in areas where the pest is established is debatable as they are known to attract more beetles to an area than they can capture (Wawrzynski & Ascerno, 1998). There is also a risk that the floral attractant may draw female beetles to areas where the pest is not known to occur. Placement of traps should therefore be considered carefully. Placement of traps by individuals not under the control of the NPPO should be discouraged or prohibited, since this may cause spread of the pest, and confuse monitoring results.

More information on the use of trapping is given in Appendix 4.

Cultural control

Cultural control methods may be used to reduce the suitability of sites for oviposition and therefore can be effective at reducing populations of *P. japonica* larvae (Allsopp, 1996). Avoiding irrigation of grassland during peak season (emergence and flight times) will aid reduction of larvae in the soil as the females seek moist sites to lay their eggs. In addition, mechanical cultivation may be effective at reducing the population of larvae by direct damage and by reducing the suitability of the habitat. Powered rotovation of the soil to a depth of at least 10 cm during dry conditions will minimize survival of larvae in soil clods. Autumn is the preferred time for this cultivation, after the peak flight season but before the larvae have burrowed deeper into the ground to overwinter.

Removal of host plants and habitats

Removal of host plants over wide areas is not recommended in California because of the risk of encouraging dispersal of the beetles and the sheer effort required (California Department of Food and Agriculture, 2014). Local destruction of heavily infested grassland (for example by ploughing) after the main flight period should reduce populations substantially. In Italy, in 2016, where larval populations were more than 50 per m^2, measures included the aeration of turf to a depth of at least 10 cm and two insecticide treatments (using chemical or biological plant protection products).

Physical removal of adult beetles

Hand removal of adults is feasible for small infestations from low-level plants and in small plantings. Switzer & Cumming (2014) evaluated hand removal at various times of the day (08:00, 14:00, 19:00) from grape vine in the USA and showed that 19:00 was the most effective time period. Ladd & Klein (1982) showed that adults can be killed following picking by dropping them into a bucket of soapy water.

Biological control

Biological control has been implemented against *P. japonica* since 1920. In the USA, 49 natural enemy species from Asia and Australia were released against the pest up until 1933 (Potter & Held, 2002). However, most biological control agents did not establish and few provided a significant level of control under field conditions. Research would be required in Europe to evaluate the potential of any biological control agents.

Paenibacillus popilliae (milky disease bacterium) has been used in the USA to control *P. japonica* larvae. Entomopathogenic nematodes (*Steinernema glaseri* and *Heterorhabditis bacteriophora*) are effective in controlling larvae in turf and nursery stock in the USA (Potter & Held, 2002).

Steinernema spp. and *Heterorhabditis bacteriophora* have been evaluated for effectiveness against *P. japonica* in the Azores (Simões *et al.*, 1993) and in Piedmont (B. Cavagna, Regione Lombardia, pers. comm., 2016). *Steinernema glaseri* and *Steinernema carpocapsae* both reduced larval populations when applied in September but proved ineffective during the colder months (November to February) (Simões *et al.*, 1993). In the Azores efforts have been made to find endemic entomopathogenic nematodes for the control of *P. japonica* (Lacey *et al.*, 2001). Surveys conducted on the islands of Terceira and Santa Maria resulted in the isolation of two *Heterorhabditis* strains with good larvicidal activity (Lacey *et al.*, 2001). None of these agents are currently commercially available within the EPPO region.

Metarhizium anisopliae were due to be trialled for treatment of infested grassland in the Ticino Valley (IT) in 2016. The fungus is applied through a modified seed drill which delivers inoculated wheat grains into slots in the sward at a spacing of 11 cm and to a depth of 5–6 cm. The results are due to be published in 2016–17.

In the USA, indigenous generalist predators (ants, staphylinids, carabids, moles and birds) are known to inflict considerable damage on populations where they feed on the eggs and early instar larvae (Potter & Held, 2002).

Chemical control

The mention of chemical products in this section does not mean they are permitted and/or available in the EPPO region. The NPPO should assess the portfolio of insecticides available to control *P. japonica* in advance of any outbreak.

Larvae

In the USA, larvae can be controlled with the application of a soil insecticide followed by watering the treated area to aid leaching of the residues into the root zone (Potter & Held, 2002). Many of the products historically available in the USA to control turf infestation are now banned due to their damage to non-target organisms. Imidacloprid and halofenozide were registered in the USA in the 1990s, and due to their persistence the products are used as preventative control of scarabaeid grubs. Immersing root balls in chlorpyrifos can control *P. japonica* larvae in nursery stock. Including bifenthrin or tefluthrin in potting media, or drenching soil with imidacloprid in the mid-summer months, can control grubs in nursery stock. Low doses of imidacloprid have been shown to have a synergistic effect with entomopathogenic nematodes by reducing the resistance of the larvae (Morales-Rodriguez & Peck, 2009). Regulatory restrictions on use of neonicotinoids and on the use of doses lower than those authorized are likely to preclude the use of this approach in many countries. Azadiractin has been used against larvae on turf (Potter & Held, 2002).

Adult beetles

In the USA, insecticide control treatment against adults has mainly been achieved by spraying above-ground parts of the host plants with insecticides. Insecticidal soap is effective against adults when it comes in direct contact with them. On the island of Terceira (Azores) spraying with chlordane, coupled with baited traps, proved insufficient to prevent the dispersal of the pest (Martins *et al.*, 1988).

Declaration of eradication

Eradication may be declared and a pest-free area re-established if *P. japonica* is found to be absent from an area based on official surveillance over at least two life cycles or at least 3 years, whichever is longer. When the pest is declared eradicated from part of a previously demarcated area, the infested area and buffer zone should be reviewed and amended accordingly.

5. Containment measures

When the presence of *P. japonica* is confirmed an evaluation of pathways should be carried out in order to determine whether other areas have already been put at risk from those pathways and what appropriate measures should be taken to reduce the risk of further spread. A survey should be carried out in any area which has been put at risk. If the area is in another country the NPPO of that country should be informed. Containment[1] measures should be taken in all cases to reduce the risk of spread, whether the strategy being followed is one of eradication or containment. Areas with high population densities of the pest must be identified within the entire infested area and specific monitoring and containment measures put in place to reduce the risk of passive dispersal.

If eradication is no longer considered to be feasible, containment measures should continue to be applied as part of a long-term strategy for management of pest risk. Containment measures taken within the demarcated zone should include:

- suppression of populations of *P. japonica* to reduce the risk of active and passive spread (methods to achieve this are described under ‘Eradication measures’ above)
- prohibition or restriction of the movement of soil and used growing medium, with or without plants, from the demarcated zone.

Vehicles

Sites representing a particular risk for passive dispersal through aircraft and vehicles can be found in:

a) car and truck parking areas
b) loading and unloading docks and surrounding areas of factories or businesses
c) refuelling stations
d) airports, heliports, bus stations, railway stations and distribution centres
e) ports and maritime container parks.

[1]Application of phytosanitary measures in and around an infested area to prevent spread of a pest [FAO, 1995]

These sites should be subject to visual monitoring. Removal of host plants, insecticide treatment of host plants and treatment of host plants with an anti-feedant such as azadirachtin may be considered to reduce the population of adult beetles within these high-risk sites. Increasing awareness through information boards and leaflets should encourage members of the public to take measures to reduce the risk of facilitating spread with their vehicles.

In exceptional cases where high numbers of beetles are already present mass trapping may be considered as a means of reducing the population. However, the risk of attracting beetles into the sites from surrounding land should be taken into account.

Other pathways

Commodities whose movement from the demarcated zone should be prohibited or restricted include:

a) all life stages of the Japanese beetle, including eggs, larvae, pupae, and adults
b) soil and used growing medium
c) plants with soil attached, including turf.

Movements of the live pest, except under license, should be prohibited. Movements of untreated soil and used growing medium out of the demarcated zone should be prohibited. Treating soil at 49°C for 15 min has been found to be effective at eliminating *P. japonica* (Government of Canada, 2015).

Movement of plants for planting

There have been few reports of the presence of *P. japonica* with plants for planting, other than in turf, but in principle they could be a significant pathway and international and internal movements of plants for planting have been regulated in other affected regions (Government of Canada, 2015).

Different categories of plants for planting in soil may be distinguished with different probabilities of pest association, in descending order:

- turf
- plants of Poaceae and Cyperaceae (grasses and sedges) in containers
- field-grown plants
- plants in large containers grown in the open
- plants in small containers grown in the open
- plants in large containers grown under protection
- plants in small containers grown under protection.

Measures

A combination of the following measures should be considered to achieve an adequate level of risk reduction:

- prohibition of movement (larvae and adults)
- movement of bare-rooted plants (larvae)
- complete physical protection (larvae and adults)
- growing and moving plants entirely outside the flight period (larvae and adults)

- physical protection of soil surface to prevent egg laying (larvae)
- movement only outside the flight period (adult beetles)
- irrigation by drip only or from below to keep soil surface dry (larvae)
- maintaining growing medium free from weeds (especially grass weeds) mechanically or by the use of herbicide (larvae)
- insecticide soil treatment (larvae)
- insecticide foliar sprays against (adult beetles)
- sampling and visual inspection of growing medium (larvae)
- visual inspection of aerial parts of plants (adult beetles).

For commodities presenting the highest risk (for example turf grass), especially in areas with a high population of the pest, prohibition of movement may be considered the only option which will reduce the risk to an acceptable level.

Acknowledgment

This Standard was first drafted by an expert working group composed of B Cavagna, M Ciampitti, D Jan Van Der Gaag, S Matthews-Berry, M Ward and R Tanner.

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Appendix 1 – Characteristics of *Popillia japonica* (all life stages) (text taken from PM 7/74 *Diagnostic Protocol for* Popillia japonica (2006))

For illustrations see photos in EPPO Global Database https://gd.eppo.int

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

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 67 spines in a characteristic V shape. 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 are 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

The adult beetle is brightly coloured 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. The male tibial spur is more sharply pointed and the tarsi are shorter and stouter than those of the female.

Appendix 2 – How to carry out visual inspections for adult beetles

Adult beetles are gregarious. Males are attracted by odour trails that advertise the presence of receptive females or host plants preferred for feeding. *Popillia japonica* adults can be detected by visual examination of the green parts of plants (EPPO, 2006). They are more visible on the vegetation during cooler times of day. They feed on leaves, consuming the soft tissues while leaving the veins untouched. Skeletonized foliage is the most common symptom associated with feeding adults, but cannot be taken as confirmation by itself, since other organisms, insects and small snails, can leave similar traces.

EPPO (2006) provides a key to Rutelidae families and the *Popillia* genus, while at the same time noting the limitations of this method (especially in the field) due to over 300 species comprising the genus *Popillia*. The widespread European native garden chafer *Phyllopertha horticola* is easily confused with *P. japonica*, having a similar lifecycle and morphological appearance. Close examination would be required to separate the two species (DEFRA, 2015).

A practical method for carrying out visual inspections in the buffer zone targeted at the parts closest to the infested zone is described below. This is the method which has been used in the Ticino Valley in Italy:

- The 3 km wide buffer zone is subdivided into three concentric bands of 1 km each in width.
- A matrix of 1 km squares is then superimposed and each square is allocated to the band in which it mostly lies.
- Within the band closest to the infested site, four sites are inspected in each square and three checks are carried out at each site, one in June, one in July and one in August.
- In the second band four sites are inspected in each square, twice, once in June and once in July.
- In the third band two sites are inspected, twice, once in July and once in August.

The interiors of wooded areas are excluded from the selection of sites within squares since the pest has not been found in such habitats.

Appendix 3 – How to sample for larvae

Sampling for larvae can be used to:

- provide evidence of the status of a place of production
- provide estimates of the population in an area before emergence.

Sampling for larvae is difficult, and is likely to be used only during the part of the year when adults are not above ground and damage in turf is evident.

General procedure

Larval populations in the soil are typically aggregated with distributions best fitted by a negative binomial distributions (Yuen-Shaung *et al.*, 1983). Samples should be taken when the majority of larvae are in the second or third instar (September–May) and preferably during spring and autumn when the larvae are nearer the surface. Samples are taken to a depth of 20 cm. The following sampling methods are approved in North America for the purpose of certifying freedom from the pest, and were also adopted in Italy in 2016:

Cup cutter or similar coring device: soil should be sampled using a cup cutter or similar coring device no smaller than 4.25 inches (12 cm) in diameter. These cup cutters are available from golf course supply companies.

Spade or shovel: soil should be sampled using a spade to extract soil 'squares' (20 cm × 20 cm × 20 cm).

Following collection, the core of soil is extracted and placed on a plastic tarpaulin or in a basin and crumbled to identify all larvae present (see Fig. 1). All larvae suspected of being beetle larvae are collected in sample tubes containing 70% alcohol and labelled with the core sample code. The test tubes are then sent to plant health laboratories for confirmation. The check sheets indicate the number of larvae found in each core sample. The number is confirmed or corrected after the laboratory has identified the larvae

Fig. 1 Sampling for *Popillia japonica* larvae in Italy. Images courtesy of Regione Lombardia (IT).

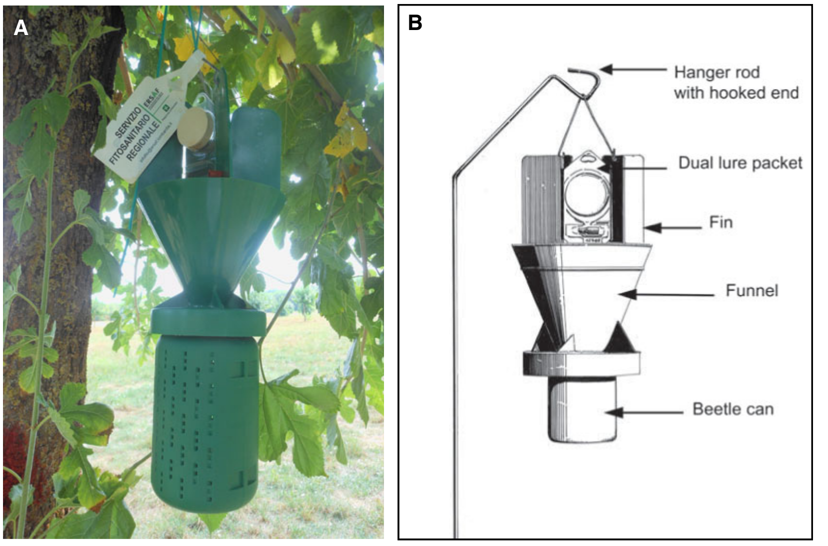

Fig. 2 (A) Standard plastic trap which is reusable with a screw-on collection container that is perforated for ventilation and drainage. The lures which come with the trap last for one season before a replacement is needed. (B) A slightly different structure (shallower beetle can) but details the different components of the trap (from California Department of Food and Agriculture, 2013).

collected. After the soil and grass sward have been examined, they are replaced in their previous position.

Sample numbers to provide evidence of the status of a place of production

Samples should be uniformly distributed across the place of production, taken within the rows in the case of field-grown nursery stock. In addition, some samples should be taken from favoured oviposition sites (for example in grass habitats at the boundaries of the site).

To provide estimates of the population in an area before emergence

Samples should be partly taken at random throughout the field, and partly targeted at the points of the field with a higher susceptibility for harbouring larvae. For example, oviposition preference is for cooler, shadier portions of fields and downwind locations, near bushes, more to the south and east than north and west. So at least half the core samples should be collected near the edge where conditions are most favourable for egg deposition. The distance between one core and the next should not be less than 20 m, unless the reduced dimensions of the field make this impossible.

In Italy in 2016, monitoring surveys consisted of 4 core samples taken for each grassland field with a surface area

Table 2. Determining the number of soil samples to collect. Table taken from Government of Canada (2015).

Block/field size in hectares (acres)	Cutter method	Spade method
0.04–0.40 (0.1–1.0)	50	20
0.44–2.02 (1.1–5.0)	70	30
2.06–4.05 (5.1–10.0)	80	35
4.08–10.12 (10.1–25.0)	90	40
10.16–20.23 (25.1–50.0)	125	50
>20.23 (50.0)	125 plus 2 samples for each additional 4.04 ha (10 acres)	50 plus 1 sample for each additional 4.04 ha (10 acres)

Note: the Canadian sampling is from a nursery management programme in order to guarantee *P. japonica*-free nursery stock (and not only to provide evidence of freedom from the pest in any kind of production place).

Fig. 3 The RESCUE! Japanese and Oriental Beetle trap which slowly releases a pheromone over 8 weeks. This trap is for use over one season and has a seal on the bottom to easily remove the contents. The trap is manufactured in the USA and costs in the region of $15.

under 0.5 ha and 6 samples are collected from fields with surface areas between 0.5 ha and 1 ha. If the surface area of the field monitored is greater than 1 ha, 2 additional core samples are collected for each extra hectare, over and above the basic 6. For example, a 4-ha field is monitored by collecting 12 core samples.

Appendix 4 – How to use traps for adult beetles

Traps (see Figs 2A,B and 3) may be used as part of detection survey, delimiting survey, monitoring of population levels within an infested area or mass trapping as an element of control. Placement of traps by individuals not under the control of the NPPO should be discouraged or prohibited, since this may cause spread of the pest, and confuse monitoring results.

Traps can attract beetles at distances of several hundred metres (Hamilton *et al*., 2007). For other species there is evidence that placing pheromone traps too close together can cause confusion and reduce the effectiveness of the traps for monitoring purposes. In the absence of specific evidence on this point for *P. japonica* it is recommended that traps for detection and delimiting surveys should be placed no closer than 200 m apart, and within the infested area for mass trapping and monitoring the population density no closer than 50 m apart.

Traps should capture about 75% of the beetles in an area (Utah State University, 2016). Other reports indicate only a 40–50% reduction in population numbers in areas with high trap density (1 per acre) (California Department of Food and Agriculture, 2014). In the USA, California (Potter & Held, 2002) and Oregon monitor 10 000 and 5000 traps per year, respectively, and have both eradicated isolated infestations in these states. In Italy, mass trapping was initiated in 2014, where 64 traps were installed and replenished every 2 weeks. Traps caught over 28 000 adults (Regione Piemonte, 2015). In the USA, mass trapping (using traps

Total traps

Core area: 1 sq. mile [2.58 sq. km] = 50 traps

1 mile buffer: 8 sq. miles [20.72 km^2] = 200 traps

2 mile buffer: 16 sq. miles [41.44 km^2] = 80 traps

3 mile buffer: 24 sq. miles [62.16 km^2] = 120 traps

Total: 49 sq. miles [126.91 km^2] = 450 traps

Delimitation survey

5	5	5	5	5	5	5
5	5	5	5	5	5	5
5	5	25	25	25	5	5
5	5	25	50	25	5	5
5	5	25	25	25	5	5
5	5	5	5	5	5	5
5	5	5	5	5	5	5

Fig. 4 Density of traps for a delimitation survey (from California Department of Food and Agriculture, 2013).

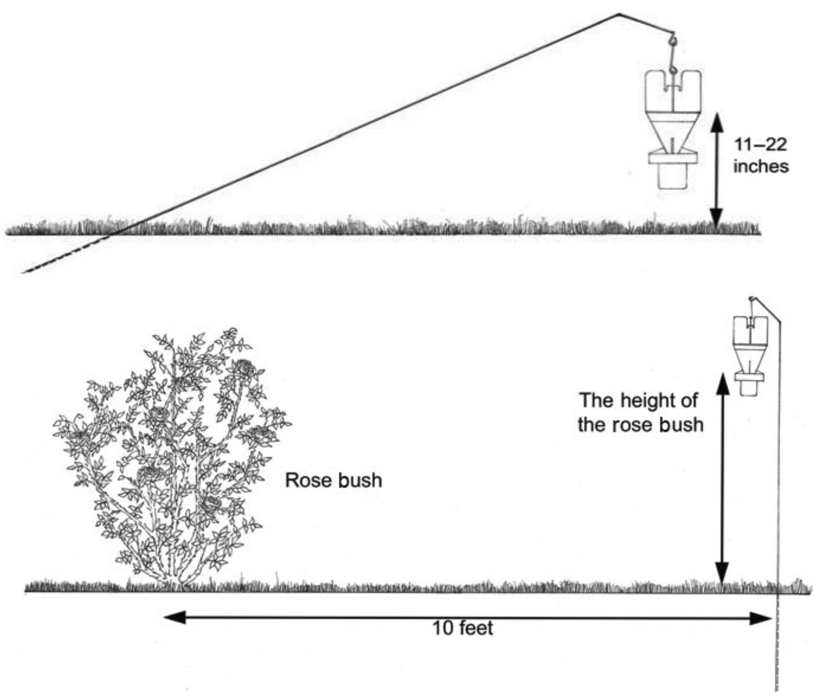

Fig. 5 Recommended positioning of traps in relation to tuft and host. Image from: California Department of Food and Agriculture (2013). Note that: 11–22 inches equates to 28–56 cm and 10 feet equates to 3 m.

baited with the Japanese Beetle Bait BiolureR System) of isolated populations over 4 years in an area bordering the Mississippi River (Minnesota) produced a 97% reduction in *P. japonica* numbers (Wawrzynski & Ascerno, 1998).

The Government of Canada (2015) state that detection survey trapping within areas where *P. japonica* is not known to occur should be conducted at the rate of one trap per 5 km^2 in areas suitable for *P. japonica* establishment. The California Department of Food and Agriculture (2013), detail that two traps per square mile (0.7 traps per km^2) should be used for detection surveys in both urban areas and rural residential areas of 300 or more homes per square mile (2.58 km^2). In addition, the author recommends that for delimitation surveys, when a beetle is trapped, densities of traps should increase in the 49 square miles (126.9 km^2) surrounding the find (see Fig. 4), with a total of 450 traps.

In high-risk sites, for example airports that receive significant travel from areas infested with Japanese beetle, the California Department of Food and Agriculture (2013) recommend that 25 traps per square mile should be placed in a 1-mile buffer zone. Trap densities around freight should be 25 to the square mile surrounding the facility.

The location of each trap should be mapped using GPS coordinates. Each trap should have a tag attached identifying it as belonging to the plant health service and bearing a code, the date of its placement and an e-mail address for more information.

Placement of traps

The California Department of Food and Agriculture (2013) provide the following information on the positioning of traps. Traps should be placed in such a position as to receive full sunlight from 10:00 to 15:00. Traps should be placed 10–25 feet (3–7.5 m) away from hosts (excluding turf). Placing the trap closer than 10 feet (3 m) or in contact with host foliage may result in beetles landing on the host plant rather than falling down into the beetle can. Traps should not be placed under foliage where debris may fall into the trap and block the funnel opening. Traps should not be placed in such a manner that will impede the safe operation of lawn-care equipment. (The edge of the lawn or just off the turf is best.) Trap placement and trap height will depend upon the hosts that are available at the selected site. When only turf or turf and a high-growing host (tree) is available, the trap height will be 11–22 inches (28–56 cm) from the funnel rim to the ground. When turf and a low-growing host (roses, grapes, etc.) are available, then the trap height will be at host level (Fig. 5).