Bulletin OEPP/EPPO Bulletin (2017) **47** (2), 164–173 ISSN 0250-8052. DOI: 10.1111/epp.12381

Diagnostics
Diagnostic

PM 7/36 (2) *Diabrotica virgifera virgifera*

Specific scope

This Standard describes a diagnostic protocol for *Diabrotica virgifera virgifera*.

This Standard should be used in conjunction with PM 7/76 Use of EPPO diagnostic protocols.

Specific approval and amendment

Approved in 2003-09.
Revised in 2017-02.

1. Introduction

The New World genus *Diabrotica* Chevrolat, 1836 is one of the largest leaf beetle genera, with about 354 described species (Derunkov *et al.*, 2015). Ten species or subspecies within this genus are generally recognized as pests (Krysan & Miller, 1986); *Diabrotica barberi* Smith & Lawrence, *Diabrotica undecimpunctata howardi* Barber and *Diabrotica virgifera virgifera* LeConte are serious pests of maize in North America, the latter species in Europe too.

There are two subspecies of *D. virgifera*, *virgifera* (western corn rootworm) and *zeae* Krysan & Smith (Mexican corn rootworm) (Krysan *et al.*, 1980). *Diabrotica virgifera virgifera* is distributed from the Mid-Western to Eastern and South-Eastern USA and northward into Ontario, Canada and is adapted to temperate climates (diapause), while *D. virgifera zeae* is distributed mainly from Texas and Oklahoma in the United States to Panama and is adapted to warm climates (without diapause). Only the subspecies *D. virgifera virgifera* has been found in the EPPO region. The pathways for introduction or spread of *D. virgifera virgifera* are infested soil containing eggs, larvae or pupae, or the aerial parts of maize plants (e.g. for fodder or green manure, or as cobs) carrying adults. In the latter case, however, the probability of spread with maize plants is low because, before harvest, adults generally move to attractive plants in other fields or soon die. Since it is likely that *D. virgifera virgifera* arrived by aeroplane, as shown by the fact that the first discovery site in Europe was in close proximity to Belgrade airport (Edwards *et al.*, 1999), adults can also probably be transported as contaminants on other means of transport (e.g. boats, trains, trucks, cars). Adult beetles can also fly up to 100 km from infested areas. Adult beetles are known to fly from mature maize onto other flowering crops. Cucurbits are particularly attractive to corn rootworms, but these pests have also been found on lucerne, clover, rape, soybean and sunflower. However, maize seems to be the preferred source of food (Toepfer *et al.*, 2015).

Further information on the biology of *Diabrotica* can be found in the EPPO data sheet on *Diabrotica barberi* and *Diabrotica virgifera* (EPPO, 1997), information on geographical distribution is available in the EPPO Global Database (EPPO, 2016).

2. Identity

Name: *Diabrotica virgifera virgifera* LeConte
Synonyms: *Diabrotica virgifera*
Taxonomic position: Insecta: Coleoptera: Chrysomelidae: Galerucinae: Luperini: Diabroticina
EPPO Code: DIABVI
Phytosanitary categorization: EPPO A2 List: no. 199, EU Annex designation: none.[1]

3. Detection

Eggs of *D. virgifera virgifera* occur in the soil down to a depth of 35 cm, most of them in the 15-cm layer (Bača *et al.*, 1995) from late summer (August/September) and throughout the winter as diapausing eggs. They occur in the soil of maize fields and, to a lesser extent, also in crops, such as soybean and cereal, in neighbouring fields (Kiss *et al.*, 2001).

[1]The EU decision 2003/766/EC on emergency measures to prevent the spread within the community of *Diabrotica virgifera* Le Conte was repealed on 6 February 2014.

Fig. 1 *Diabrotica virgifera virgifera*, third larval instar.

Larvae generally occur between May and early August with a peak from May to June (Bača *et al.*, 1995).

There are three larval instars which live in the soil near maize roots. Grassy weeds (e.g. *Setaria* spp.) can serve as alternative larval hosts in maize fields (Moeser & Vidal, 2004). The highest number of larvae is found to a depth of 15 cm (Bača *et al.*, 1995). The main damage is caused to the root system. The third larval instar (Fig. 1) is mainly responsible for most damage to the root system, and this damage is commonly referred to as 'root pruning' (Fig. 2).

Larvae pupate in soil chambers in the root zone. They remain in the pupal stage for 7–10 days (Sivčev *et al.*, 2012).

Adults of *D. virgifera virgifera* are found from the end of June to mid-October with a peak in the second half of July and during August. Adults most commonly occur on the leaf, tassel and silks (i.e. the leaf axil and ear tip of maize) before, during and after flowering, respectively (Bača *et al.*, 1995).

Pheromone traps (e.g. PALs) (Fig. 3) or yellow sticky traps are used to catch the adults. These should be placed in maize fields or nearby in June. Traps should be checked at least every 2 weeks, but preferably each week, up to the end of September. The distance between the pheromone

Fig. 2 *Diabrotica virgifera virgifera*, 'root pruning' damage.

Fig. 3 Pheromone trap.

traps should not be less than 20 m. They should also be placed at points of entry, airports, ports and transhipment locations (including army barracks, if there is a risk of movement of army equipment from infested areas) in order to monitor introductions. Host plant volatiles from both maize and cucurbit blossoms and some floral odorants attract adults (Hammack & Petroski, 2004).

European beetles that are commonly present in the vicinity of corn fields and that might be found in traps are presented in Fig. 4. Beetles that are regularly found in traps and that can be confused with *D. virgifera virgifera* when in poor condition are presented in Fig. 5.

4. Identification

Identification is usually based on morphology. Although identification should be performed on adults, information is also provided on eggs and larvae.

4.1 Morphological identification

4.1.1 Description of the genus Diabrotica

The species in *Diabrotica* have an elongate, oval body, with the prothorax being narrower than the elytra (Derunkov *et al.*, 2015). The basic colour of the beetles may be green or yellow, usually with spots, vittae or bands. The colour patterns in most Central American species are bright and variegated, and frequently iridescent. The head has short genae that are no longer than a quarter of the diameter of the eye. The antennae are long, filiform, rarely slightly serrate, and in males frequently modified, with the middle antennomeres being thickened or bearing a longitudinal costa on the inner side; antennomeres 2 and 3 are subequal in length, or antennomere 3 is slightly longer, but usually no more than 1.5 times longer than antennomere 2. The pronotum is quadrate or subquadrate, usually with the ratio of width to length being 1.23–1.37; only rarely is the pronotum slightly transverse with the ratio of width to length exceeding 1.38. The pronotum is either with or without two discal

Fig. 4 European beetles that are in the vicinity of corn fields (left to right): *Cryptocephalus vittatus*, *Cryptocephalus decemmaculatus* and *Phyllobrotica quadrimaculata.*

Fig. 5 Beetles regularly found in traps that could be confused with *Diabrotica virgifera virgifera* when in poor condition (left to right): *Demetrias atricapillus* (Coleoptera: Carabidae), *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae), *Rhagonycha fulva* (Coleoptera: Cantharidae).

depressions; when present, the discal depressions may be shallow, wide or deep, and they are sometimes represented by a small, round fovea. The pronotum surface is smooth, shining or shagreened. Shagreening may be represented by narrow mesh, minute wrinkles or minute tubercles. The elytra lack notches, although there are sometimes one or two depressions on each elytron; they may be sulcate with 2–5 distinct sinuate sulci, and they are usually distinctly shagreened. The elytral punctures are irregularly arranged. The legs are not modified. Males have apical spurs on the middle and posterior tibiae, but none on the anterior. Females have spurs on all tibiae. The front coxal cavities are open. Claws are bifid. The last ventral abdominal segment in males is slightly truncate, without an apical lobe. The aedeagus is symmetrical, or rarely asymmetrical with a cavity on the right side, and it is without basal spurs; the orifice is covered by a sclerotized plate; and the internal sac has a complex armament consisting of robust, chitinized hooks or plates, with 2–6 sclerites.

4.1.2 Description of the species Diabrotica virgifera

4.1.2.1 Eggs. White, yellowish to brown and 0.5 mm long. The identification of the species from the eggs is difficult without the aid of a scanning electron microscope. At a magnification of 1200 times, the eggs are distinguished by the external sculpturing of the chorion. More details are given by Krysan & Miller (1986).

4.1.2.2 Larvae. Larvae can only be identified with certainty at the subfamily level based on external morphological characters and reference to the host plant(s) (Lawrence, 1991). Observation should be made on the third larval stage using a stereomicroscope with a minimum magnification of 40× and several specimens should be observed to take account of any polymorphism. A dissection of the cephalic capsule is necessary for examination of the mandibles.

Chrysomelid larvae can be distinguished by the following characters:

- mandibles palmate and toothed, without mola (Fig. 6)
- legs generally well developed, usually with 5 segments, including tarsus and pretarsus that are fused to form a tarsungulus (Fig. 7).

Larvae of subfamily Galerucinae can be identified by combination of the following characters:

- 2 antennal segments (Fig. 8)
- 0 or 1 pair of stemmata (Fig. 9) (no stemmata in *D. virgifera*, Fig. 1)
- presence of 4–5 teeth on the mandibles (Fig. 6) (5 teeth for *D. virgifera*).

The larvae can be confused with other European soil-dwelling insect species (such as wireworms), which can be

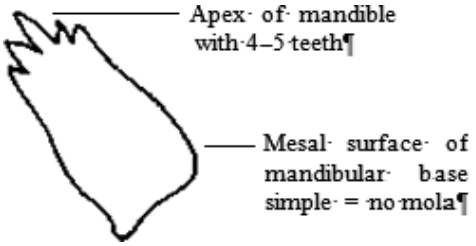

Fig. 6 Mandible.

Fig. 7 Leg.

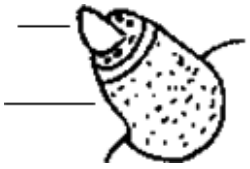

Fig. 8 Antenna.

found at the same time on maize roots. The *D. virgifera virgifera* larva is elongated and 10–18 mm long in the third larval stage (total of three larval stages). The larva is white to yellowish with a light-brown head capsule and a brown plate on the dorsal side of its posterior end (Fig. 1). Wireworm (*Agriotes* spp.) larvae are longer, up to 20–25 mm and brilliant yellow (*Agriotes lineatus*) to yellow-brown (*Agriotes obscurus*). The head capsule of a wireworm larva is dark brown and the entire cuticle of the insect is much more rigid than that of *D. virgifera virgifera*.

Besides *D. virgifera*, larvae of *Diabrotica undecimpunctata howardi* and *Diabrotica longicornis* can feed on maize roots. The anal plate or pygidial shield on the ninth abdominal segment can be used to differentiate mature larvae (alive or stored in 70% ethanol). Furthermore, the dark lines on head capsules give additional hints for the differentiation of the species (Mendoza & Peters, 1964). Urogomphi (varying considerably in size) are present in *D. undecimpunctata* (Fig. 10-1B) but not in *D. virgifera* (Fig. 10-2B) or *D. longicornis* (Fig. 10-3B). The postventral margins of the anal plates of *D. undecimpunctata* (Fig. 10-1B) and *D. virgifera* (Fig. 10-2B) are dark brown in contrast to *D. longicornis* (Fig. 10-3B). A typical character for the species *D. virgifera* is the darkened area with a notch on the anterior margin of the anal plate (Fig. 10-2B). Furthermore, the area underneath the angle of the posterior edge of the anal plate has a narrow sclerotized band in *D. undecimpunctata* and *D. virgifera* but not in *D. longicornis* (Mendoza & Peters, 1964).

For further verification of the species, the head capsule of *D. undecimpunctata* has a cross-like, dark but diffused pattern on the median region, with arms terminating at the base of two laterally situated setae (Fig. 10-1B), in contrast to *D. virgifera* and *D. longicornis*. A typical character of *D. virgifera* is the dark band which continues anteriorly along the cranial suture and for about one-third the length of each frontal suture, creating a bifurcate pattern (Fig. 10-2A). This bifurcate pattern is more diffused than the marginal band (Mendoza & Peters, 1964).

The key (Table 1) of Mendoza & Peters (1964) can be used in combination with Fig. 10 to differentiate mature larvae of *D. virgifera* from those of *D. undecimpunctata howardi* and *D. longicornis*.

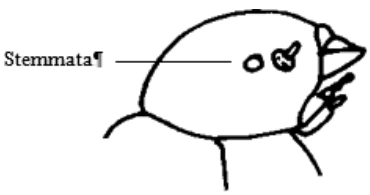

Fig. 9 Head.

Table 1. Key to mature larvae of *Diabrotica* species

1.	Rootworm larvae with urogomphi, head capsule side definitely rounded, and cross-like, diffuse marking on median region of frons (Fig. 10-1A, 10-1B)	*D. undecimpunctata*
	Rootworm larvae without urogomphi, head capsule side almost straight and elongate, and with dark lines along median region of frons but lacking cross markings	2
2.	Larvae with definite notch in darkened area of anterior margins of anal plate and with sclerotized band underneath posterior edge of anal plate. Also, a dark band along cranial suture extending ⅓ length of frontal (Fig. 10-2A, 10-2B)	*D. virgifera*
3.	Larvae with rounded anterior margins of the darkened area of anal plate and lacking sclerotized ventral band; also with dark band along cranial suture but lacking along frontal sutures (Fig. 10-3A, 10-3B)	*D. longicornis*

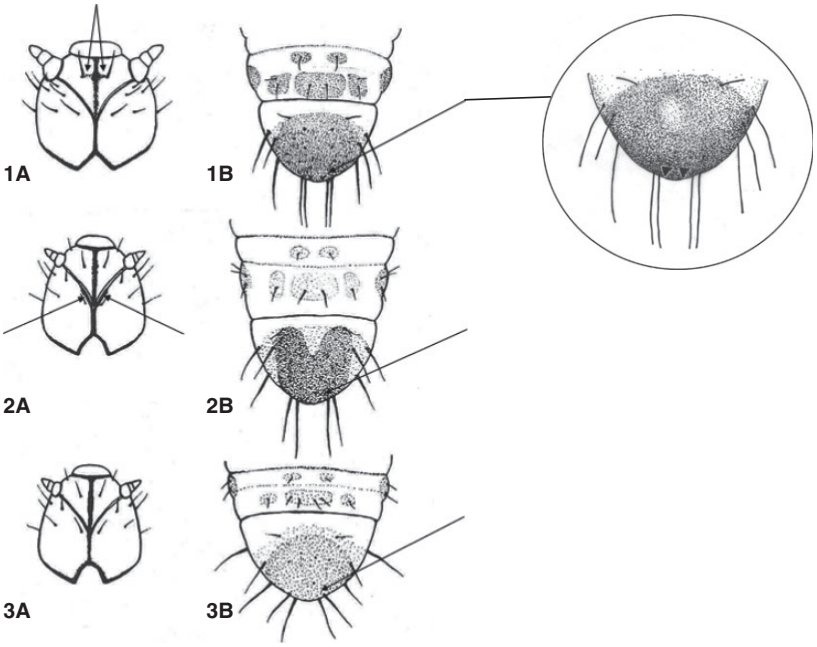

Fig. 10 Head capsule (A) and anal segments (B) of *Diabrotica undecimpunctata horwardi* (1A, 1B), *Diabrotica virgifera* (2A, 2B) and *Diabrotica longicoris* (3A, 3B).

4.1.2.3 Adults

For a positive identification, adult specimens should be examined with a stereomicroscope with a minimum magnification of 40× and some small characters should be observed with magnification of 60× or more. Whenever possible, several specimens should be observed to take account of any polymorphism. The specimen(s) should show the characteristics of the Chrysomelidae, Galerucinae, of the genus *Diabrotica,* species *virgifera* and subspecies *virgifera* as described below.

When adults are damaged, the observation of male genitalia may be needed for a positive identification.

Additional information on *D. virgifera virgifera* and on other species is also provided by the USDA APHIS PPQ Centre for Plant Health Science and Technology, USDA Agricultural Research Service, University of Maryland, and Louisiana State University (http://idtools.org/id/beetles/diabrotica/ [09.30.2013]).

Adult Chrysomelidae have (Delvare & Aberlenc, 1989):

- cryptopentamera tarsi (4th article very small and hidden within the bi-lobed 3rd; Fig. 11)
- antenna equal to or a little longer than the body
- base of antennae not surrounded by the eyes and not inserted on a head projection.

Adult Galerucinae have:

- antennae on the median part of the face, very close together (Fig. 12)
- noncylindrical body (Fig. 13)
- head directed forwards (Fig. 12).

Adult *D virgifera* have:

- habitus more elongate than convex
- size between 4.2 and 6.8 mm (female) and 4.4 and 6.6 mm (males)

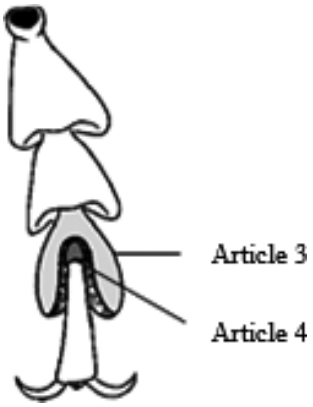

Fig. 11 Tarsus.

- head shiny black, width of genal space less than a quarter of the maximal diameter of the eye
- antennae moniliform, slightly longer than the body with sparse, moderately long, suberect straight setae. The antennae of the male are longer than those of the female. Pubescence sparse or absent on segment 1, sparse on segments 2 and 3, dense on segments 4–11; segments 1, 2 and 3 smooth, shinning, remaining segments scabrous; segments 4–11 of approximately equal diameter; in males antennal segments 2 and 3 are equal in length; in females antennal segment 3 varies from slightly longer than, to 2 times longer than, segment 2; antennal segments 2 and 3 together more than ½ the length of segment 4
- pronotum subquadrate, bifoveate, pale yellow, sometimes with orange markings, shining, very weakly and sparsely punctulate; glabrous except for long setae on the anterior and posterior angles; 1 or 2 short setae on the lateral

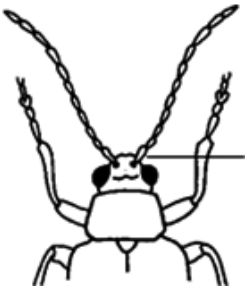

Fig 12 Anterior part of the body.

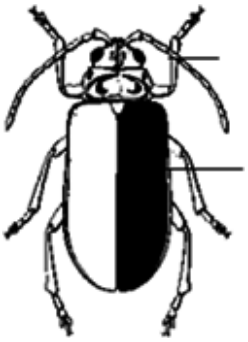

Fig. 13 General aspect of a Chrysomelidae.

margin adjacent to the long setae and several setae directed posteriorly along the posterior margin; setae at the angles extremely fragile and hence frequently missing; lateral margin distinctly deflexed

- elytra with a longitudinal carina parallel or nearly, extending to at least two-thirds of the elytra (Figs 14 and 15). Black vittae extending from humeral angles and on the suture, mostly by the females (Fig. 15), often covering most of the elytra. Males are in general darker than females (Fig. 16)
- the femora are black or pale with the outer edge coloured with black; the tarsi are black (Figs 17 and 18)

Adults of Diabrotica virgifera virgifera—The adult females are 4.2–6.8 mm long and the adult males 4.4–6.6 mm long. The body (elytra and pronotum) is pale yellow. The femora are black or pale with the outer edge coloured with black and the tarsi are black. The elytra have longitudinal carinae on the disc. Elytra are with marginal black vittae (longitudinal stripes on each side) and on the suture, mostly in the females (Fig. 15), often covering most of the elytra. Males are in general darker (Fig. 16) than females (Fig. 15). It is more reliable to determine sex by comparing the apex of the abdomen. The males have an additional sclerite on the apex of the abdomen and the abdomen has a rather blunt apex, whereas that of the female is pointed (Fig. 19). Moreover, the antennae of the male are longer than those of the female.

For the adult, there is little possibility of confusion with European species (Mohr, 1966). The European chrysomelids

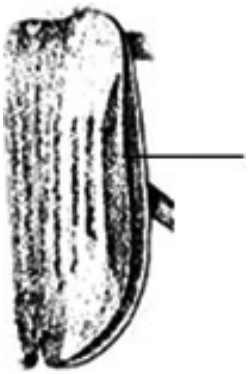

Fig. 14 Right elytra.

Fig. 15 *Diabrotica virgifera virgifera* adult female. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig. 16 *Diabrotica virgifera virgifera* adult male.

Cryptocephalus vittatus Fabricius, *Phyllobrotica quadrimaculata* Linnaeus and *Xanthogaleruca luteola* (Muller) show some resemblance in size, shape and basic colour, but colouring on the pronotum and elytra is a salient characteristic. Furthermore, these 'similar' species are not usually observed in maize fields, although they may be seen on some wild plants near these fields. *Diabrotica virgifera virgifera* may also be found on such wild plants.

Fig. 17 Femur.

Fig. 18 *Diabrotica virgifera virgifera* femur. [Colour figure can be viewed at wileyonlinelibrary.com]

Male genitalia—When adults are damaged, examination of the male genitalia is needed for a positive identification. Details are provided below:

- male genitalia (aedeagus) as illustrated in Figs 20–22.
- internal sac of the aedeagus bears 4 sclerites (Figs 21 and 22).

The shape of the aedeagus is symmetric (Fig. 20). The internal sac bears 4 sclerites (Figs 21 and 22); sclerite 1 is elongate with a rounded apical plate frequently toothed on the apical and lateral margins and bearing a lateral arm directed basally (may be not readily visible); sclerite 2 is elongate, flat and saw-like, toothed laterally, usually toothed apically and lies medially, basal of sclerite 1; sclerite 3 is small, fan-shaped, frequently toothed and lies at the base of the internal sac; sclerite 4 is an elongate, gently arcuate, apically directed spine adjacent to sclerite 2.

Separation from species not present in the EPPO region—In North America, 9 species or subspecies of *Diabrotica*, are generally considered as pests, in addition to *D. virgifera virgifera*: *Diabrotica adelpha* Harold, *Diabrotica balteata* LeConte (banded cucumber beetle), *Diabrotica barberi* Smith & Lawrence (northern corn rootworm), *Diabrotica speciosa speciosa* Germar, *Diabrotica speciosa vigens* Erichson, *Diabrotica undecimpunctata undecimpunctata* Mannerheimer (western spotted cucumber beetle), *Diabrotica undecimpunctata howardi* Barber (southern corn rootworm), *D. virgifera zeae* Krysan & Smith (Mexican corn rootworm) and *Diabrotica viridula* (Fabricius). The key of Krysan & Miller (1986) can be used for differentiation of the adults of 13 *Diabrotica* species which occur in US agriculture. This key (Table 2, Fig. 23A–I) also takes account of the distribution in North America.

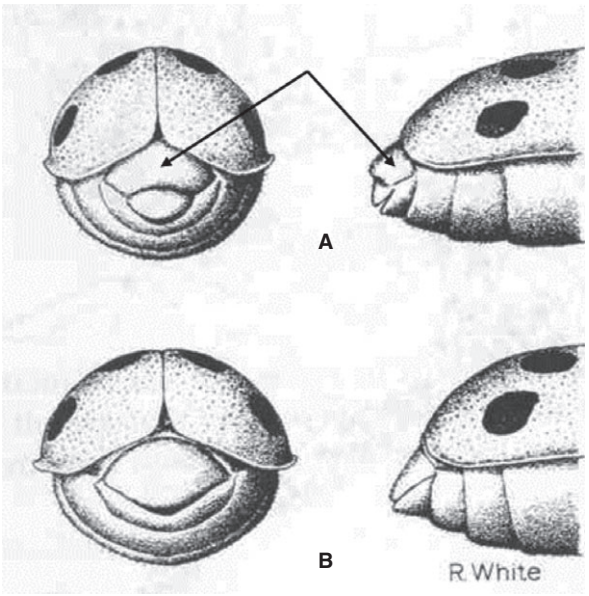

Fig. 19 Differentiation of male (A) and female (B) of *Diabrotica* spp.: Males have an additional sclerite (arrows) on the apex of the abdomen and the abdomen has a rather blunt apex, unlike the female.

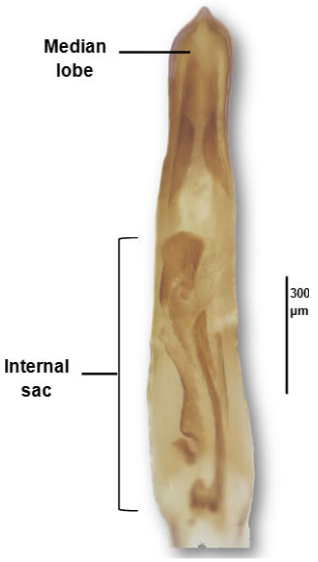

Fig. 20 Median lobe and internal sac of the aedeagus of *Diabrotica virgifera* in ventral view.

The only other North American chrysomelid that is similar to *D. virgifera virgifera* is *Acalymma vittatum* (Fabricius), the striped cucumber beetle (Fig. 24), which is not present in Europe. *Acalymma vittatum*, like *D. virgifera virgifera*, feeds on flowers and fruits of cucumbers and can be misidentified.

4.2 Molecular identification

A protocol for DNA barcoding based on COI is described in Appendix 1 of PM 7/129 *DNA barcoding as an identification*

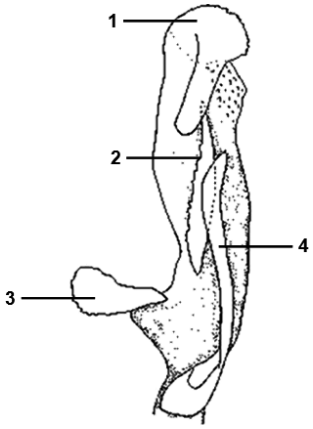

Fig. 21 Example of internal sac of the aedeagus of a *Diabrotica* spp. in ventral view with four sclerotized plates (numbered from 1 to 4).

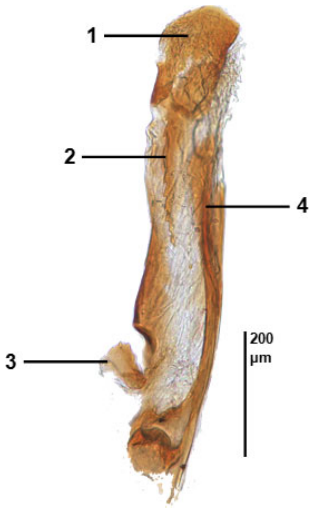

Fig. 22 Internal sac of the aedeagus of *Diabrotica virgifera* in ventral view. Sclerotized plates (numbered from 1 to 4).

tool for a number of regulated pests: DNA barcoding of arthropods (EPPO, 2016) and can be used in support of the identification; however, validation data is not provided in this Standard for *D. virgifera virgifera*. Sequences are available in Q-bank (http://www.q-bank.eu/Nematodes/), NCBI (https://www.ncbi.nlm.nih.gov/) and BOLD (http://boldsystems.org/).

Biochemical methods (isozyme electrophoresis and histochemistry) can be used to distinguish all larval stages of *D. virgifera virgifera* and *D. barberi* (Krysan & Miller, 1986). However, the use of these methods is not very practical in large-scale investigations. In Europe, *D. virgifera virgifera* is the only species present, so there is no need for separation of species at the moment.

5. Reference material

Reference material is available from most laboratories in the EPPO region (see http://dc.eppo.int).

Table 2. Key to the adults of 13 *Diabrotica* species which occur in US agriculture

1	Elytra in part pale, yellow or green	2
	Elytra entirely dark; 4–6 mm long	*D. cristata*
2	Pronotum pale, yellow or green	3
	Pronotum black (montane grasslands of New Mexico, western Texas and Arizona)	*D. lemniscata*
3	Elytra with longitudinal carinae on the disc	4
	Elytra lacking discal carinae	8
4	Femora black or pale with outer edge tinged with black	5
	Femora green or yellow; not marked with black	6
5	Elytra with black vittae from humeral angles and on the suture, often covering most of the elytra; beetle usually yellow and black	*D. virgifera virgifera* (Fig. 23A)
	Elytra entirely pale or with a narrow piceous vitta extending posteriorly from the humeral angle; beetle largely green	*D. virgifera zeae* (Fig. 23B)
6	Antennae (except proximal segments), clypeus, tibiae, and tarsi infuscated, often black	7
	Antennae clypeus, tibiae, and tarsi, yellow or testaceous; humeral vittae if present very weakly coloured	*D. barberi* (in part) (Fig. 23C)
7	Scutellum usually black (Nebraska and Texas to Colorado and Arizona)	*D. longicornis*
	Scutellum yellow or testaceous; Pennsylvania and Quebec to Vermont and Massachusetts; populations in Georgia and South Carolina have a black scutellum	*D. barberi* (in part)
8	Elytra with black markings	9
	Elytra without black markings; green with yellow transverse bands	*D. balteata* (Fig. 23D)
9	Elytra with 11 black spots	10
	Each elytron with a basal black area enclosing a pale spot and an arcuate transverse black band in the apical third	*D. tibialis*
10	Legs and abdomen entirely black (Pacific Coast)	*D. u. undecimpunctata*
	Abdomen pale, legs in part pale	11
11	Spots black and rather large; form robust (eastern US)	*D. u. howardi* (Fig. 23E)
	Spots smaller and brownish; form less robust	*D. u. tenella*

6. Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

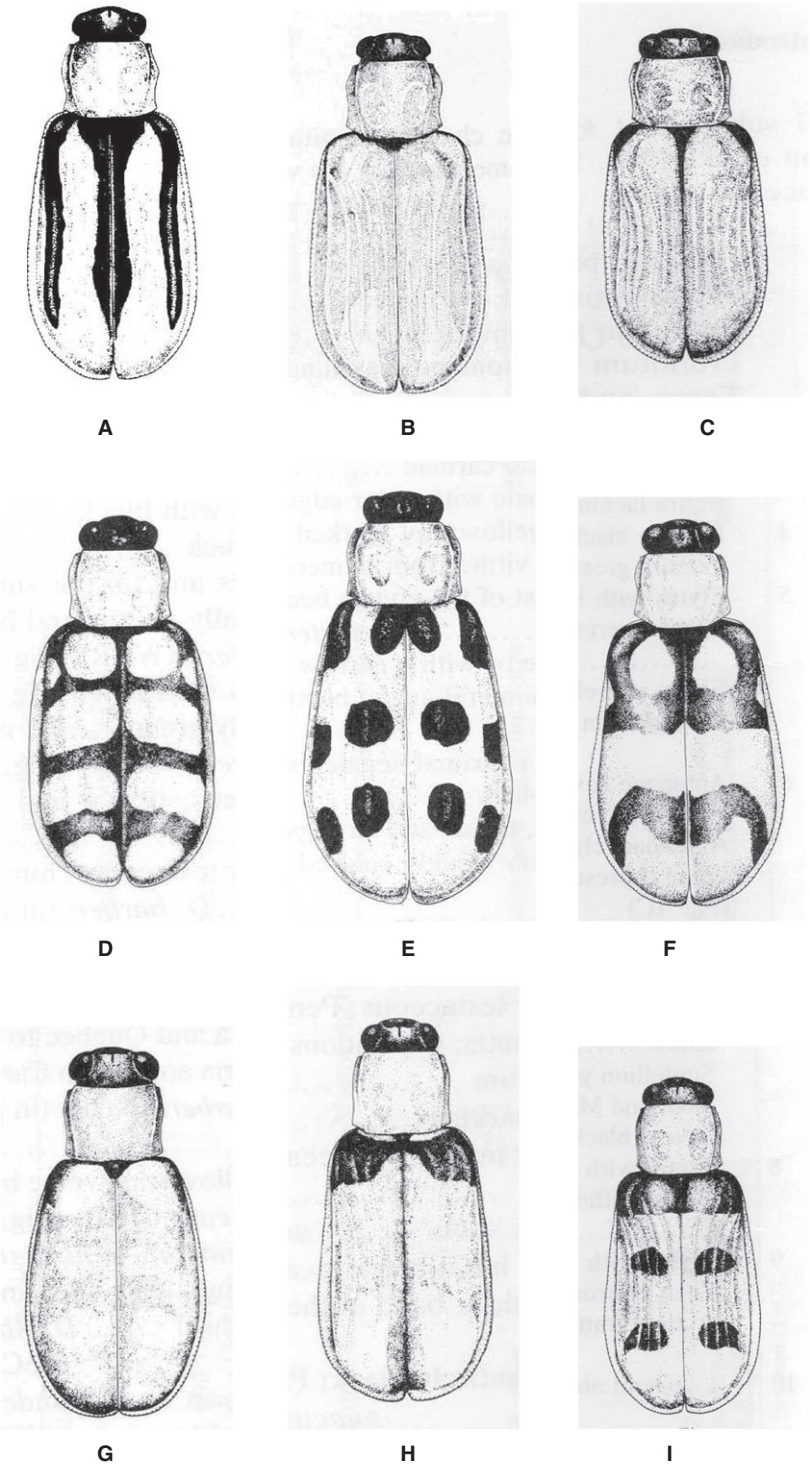

Fig. 23 (A) *Diabrotica virgifera virgifera* (western corn rootworm), (B) *Diabrotica virgifera zeae* (Mexican corn rootworm), (C) *Diabrotica barberi* (northern corn rootworm), (D) *Diabrotica balteata* (banded cucumber beetle), (E) *Diabrotica undecimpunctata howardi* (southern corn rootworm), (F) *Diabrotica adelpha*, (G) *Diabrotica speciosa*, (H) *Diabrotica viridula* (variation), (I) *Diabrotica viridula* (variation).

7. Performance criteria

When performance criteria are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

Fig. 24 *Acalymma vittatum* (courtesy John R. Maxwell).

8. Further information

Further information on this organism can be obtained from: C. R. Edwards, Purdue University, Department of Entomology, 1158 Smith Hall, West Lafayette, IN 47907-1158 (US).

9. Feedback on this diagnostic protocol

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share, please contact diagnostics@eppo.int.

10. Protocol revision

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

11. Acknowledgements

This protocol was originally drafted by: P. Baufeld, Julius Kühn-Institut (JKI), Federal Centre for Cultivated Plant, Institute for National and International Plant Health, Kleinmachnow (DE); P. Reynaud, ANSES-LSV, Entomology and Invasive Species Unit, Montpellier (FR).

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