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Organisation Européenne et Méditerranéenne pour la Protection des Plantes
European and Mediterranean Plant Protection Organization

Normes OEPP
EPPO Standards

Diagnostic protocols for regulated pests
Protocoles de diagnostic pour les organismes réglementés

PM 7/36

Organization Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations

Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

Scope

EPPO Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are written according to a 'common format and content of a diagnostic protocol' agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use, etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all diagnostic protocols:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable
- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

References

EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.

FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).

IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).

IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).

OEPP/EPPO (2003) EPPO Standards PM 1/2 (12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris.

Definitions

Regulated pest: a quarantine pest or regulated non-quarantine pest.
Quarantine pest : a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

Outline of requirements

EPPO Diagnostic Protocols for Regulated Pests provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

Existing EPPO Standards in this series

Nineteen EPPO standards on diagnostic protocols have already been approved and published. Each standard is numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:

PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44

PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51

PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60

PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69
PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77
PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253
PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259
PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265
PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275
PM 7/10 (1) *Cacyreus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279
PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292
PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298
PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310
PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256
PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264
PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270
PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280
PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288
PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296

Several of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four 'contractor' diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 'intercomparison' laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostic protocols for regulated pests[1]
Protocoles de diagnostic pour les organismes réglementés

Diabrotica virgifera

Specific scope

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

Specific approval and amendment

Approved in 2003-09.

Introduction

The genus *Diabrotica* includes approximately 338 species in the world (Wilcox, 1972), of which 10 species or subspecies are generally recognized as pests (Krysan & Miller, 1986). *Diabrotica virgifera*, together with *D. barberi* and *D. undecimpunctata howardi*, are serious maize pests in North America. In the USA, it has been estimated that the financial loss due to corn rootworms in terms of treatment costs and crop losses amounts to 1000 million USD annually (Krysan & Miller, 1986). Corn rootworm infestations have been shown to decrease yields of maize by 10–13% (Apple *et al*., 1977; Petty *et al*., 1968). Adult beetles are known to fly from mature maize onto other flowering crops. Cucurbits are particularly attractive to corn rootworms (Web Fig. 7), but these pests have also been found on lucerne, clover, rape, soybean and sunflower. In Europe (EPPO/CABI, 1997) *D. virgifera* was first observed in 1992 near the city of Belgrade (YU) (Baca *et al*., 1995). Its dispersal from Serbia has primarily been to the west, north-west, north-east and east. It is currently found in Austria, Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Hungary, Romania, Slovakia & Ukraine. Since 1998, there have been three outbreaks of this insect near the airports of Venezia (1998) and Milano (2000) (IT), and near the airport of Lugano-Agno (CH) 2000 and in France (2002). These populations are currently under eradication. There are two subspecies of *D. virgifera*, *virgifera* (western corn rootworm) and *zeae* Krysan & Smith (Mexican corn rootworm) (Krysan *et al*., 1980). *D. v. virgifera* is distributed from the Midwestern to eastern and south-eastern USA and northward into Ontario (CA) and is adapted to temperate climates (diapause), while *D. v. zeae* is distributed mainly from Texas and Oklahoma (US) to Panama and is adapted to warm climates (without diapause). Only subsp. *virgifera* has been found in the EPPO region. The pathways for introduction or spread of *D. 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* arrived by aeroplane, as evidenced by the fact that the first discovery site in Europe was in close proximity to Belgrade airport in Yugoslavia (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.

Identity

Name: *Diabrotica virgifera* LeConte
Taxonomic position: Insecta: Coleoptera: *Chrysomelidae*: *Galerucinae*: *Luperini*: *Diabroticina*
Bayer code: DIABVI
Phytosanitary categorization: EPPO A2 list: no. 199, EU Annex designation: I/A1

Detection

Eggs of *D. virgifera* occur in the soil down to a depth of 35 cm, most of them in the 15 cm-layer (Baca *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 in neighbouring fields, such as soybean and cereal (Kiss *et al*., 2001).

Generally, the larvae occur between May and early August with the peak from May to June (Baca *et al*., 1995). There are three larval stages which live in the soil near maize roots (Web

[1]The Figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

Fig. 1). The highest number of larvae are found to a depth of 15 cm (Baca *et al*., 1995). The main damage is caused to the root system of the plant by the larvae, especially the third larval stage, and this damage is commonly referred to as 'root pruning' (Web Fig. 2). The pupal stage is very short-lived.

Adults of *D. virgifera* (Web Fig. 3) 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. leaf axil and ear tip of maize) before, during and after flowering, respectively (Baca *et al*., 1995).

Pheromone or MCA (p-methoxy-cinnamaldehyde) traps (yellow sticky traps) are used for catching the adults. These should be placed in the maize fields or nearby in June. The check should be repeated at least every two weeks, but preferably each week, up to the middle of September. The distance between the pheromone traps should not be closer than 20 m because of disruption of males. 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.

Identification

Description of the genus Diabrotica

Head not inserted into the pronotum up to the hind margin of the eyes, frons with a fine longitudinal furrow (length and depth variable according to species), vertex foveolate, a longitudinal carina between the antennae. Eyes widely oval, entire, labrum truncated or weakly marginate. Antennae 11-segmented, slender, filiform, at least as long as half of the body, sometimes longer than the body. Second and third antennal segments are often very small. Maxillary palp 3-segmented with the terminal article conical, shorter and narrower than the previous one. Thorax slightly broader than long, sometimes almost square, sides nearly parallel, somewhat sinuate, disc generally bifoveolate. Thorax weakly oval posteriorly. Elytra with a distinct lateral margin, somewhat sinuate, epipleuron distinctly differentiated up to the apex; anterior coxa contiguous, prosternum with a single linear prosternal process, the cavities open posteriorly; long metaepisternum, narrows back. Legs moderately long and slender, tibias slender, mid and hind tibias with a terminal spur, outer edge with a carinae from the knee to the end, except in some species; first hind tarsal segment at least as long as the other two, sometimes as long as the next three; bifid claws. Fore coxal cavities open. Tibia little or not sulcate, claws sometimes simple (Monoxia) (Christensen, 1943).

Description of the species Diabrotica virgifera

Eggs

White to yellowish 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).

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). The 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 for the observation of the mandibles is necessary.

Chrysomelid larvae can be distinguished by the following characters:

- mandibles palmate and toothed, without mola (Web Fig. 8)
- legs generally well developed, usually with 5 segments, including tarsus and pretarsus that are fused to form a tarsungulus (Web Fig. 9).

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

- 2 antennal segments (Web Fig. 10)
- 0 or 1 pair of stemmata (Web Fig. 11) (no stemmata in *D. virgifera*)
- presence of 4–5 teeth on the mandibles (Web Fig. 8) (5 teeth for *D. virgifera*).

Biochemical methods (isozyme electrophoresis and histochemistry) can be used to distinguish all larval stages of *D. 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* is the only species to have been identified, so there is no need for separation of the species.

Adults

Adults should be observed with a stereomicroscope with a minimum magnification of 40 ×. Several specimens should be observed to take account of any polymorphism. The adult females are 4.2–6.8 mm and the adult males are 4.4–6.6 mm long. The body (elytra and pronotum) is pale yellow. The femora are black or pale with outer edge coloured with black and the tarsi are black. The elytra have longitudinal carinae on the disk. Elytra are with black vittae from humeral angles and on the suture, often covering most of the elytra. Males are in general darker than females. 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. Also, the antennae of the male are longer than those of the female.

Adult Coleoptera have:

- front wings hardened to form elytra, body usually very sclerified (Web Fig. 12)
- antennae usually of 11 segments (Web Fig. 12).

Adult Chrysomelidae have (Delvare & Aberlenc, 1989):

- cryptopentamera tarsi (4th article very small and hidden within the bilobed 3rd, Web Fig. 13)
- antenna equal or 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 (Web Fig. 14)

- noncylindrical body
- head directed forwards (Web Fig. 14).

Adult *Diabrotica* have:

- elytra with a longitudinal carina (Web Fig. 15)
- moniliform antennae slightly longer than the body
- yellow femurs with black front margin (Web Fig. 16)
- black head
- pronotum pale yellow, sometimes with orange markings
- size between 4.2 and 6.8 mm.

The taxonomy of this large genus is in a confused state largely because the taxa are very difficult to distinguish by external characters, and the only available reviews are from the 19th century and cover restricted geographic areas.

The definition of the *D. virgifera* group was based on certain external characteristics (Seeno & Wilcox, 1982):

- small to moderate size
- habitus more elongate than convex
- pronotum subquadrate, bifoveate, 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 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 distinct humeral plicae, parallel or nearly, extending to at least 2/3 of the elytra; usually 2 or more distinct sinuate discal sulci are sometimes obsolete in some specimens; elytral setae short, suberect to erect, sparse on apical margin, extremely sparse on disc
- head shining, width of genal space less than 1/4 the maximal diameter of the eye
- antennae with sparse, moderately long, suberect straight setae; subappressed slightly arcuate 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 1/2 the length of segment 4
- male genitalia as illustrated in Web Fig. 17
- internal sac of the aedeagus bears 4 sclerites (Web Fig. 18) sclerite 1 is a rounded apical plate frequently toothed on the apical and lateral margins and always bearing a lateral arm or lobe directed basally; 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, just basal of sclerite 2; sclerite 4 is an elongate, gently arcuate, apically directed spine originating from a cage-like structure at the base and ending near the lateral arm of sclerite 1 and adjacent to sclerite 2; in overall arrangement of the aedeagus, sclerites 2 and 3 are ipsilateral, as are sclerites 4 and that side of sclerite 1 bearing the basally directed lateral arm.

Possible confusion with similar species

The larvae can be confused with other European soil-dwelling insect species (such as wireworms), which can be found at the same time on maize roots. The *D. 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. Wireworm (*Agriotes* spp.) larvae are longer, up to 20–25 mm and brilliant yellow (*A. lineatus*) to yellow-brown (*A. 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*.

For the adult, there is little possibility of confusion with European species (Mohr, 1966). Only the chrysomelids *Acalymma vittatum* (Fabricius), *Coptocephala unifasciata* Scopoli, *Cryptocephalus decemmaculata* Linnaeus, *Cryptocephalus moraei* Linnaeus, *Cryptocephalus vittatus* Fabricius, *Phyllobrotica quadrimaculata* Linnaeus and *Pyrralta luteola* (Muller) show some resemblance in size, shape and in basic colour, but colouring on the pronotum and elytra are salient characteristics. Furthermore, these 'similar' species are not usually observed in maize fields, although they may be seen on some wild plants near these fields. *D. virgifera virgifera* may also be found on such wild plants.

In North America, 9 species or subspecies of *Diabrotica*, in addition to *D. virgifera virgifera*, are generally recognized as pests: *D. adelpha* Harold, *D. balteata* LeConte (banded cucumber beetle), *D. barberi* Smith & Lawrence (northern corn rootworm), *D. speciosa speciosa* Germar, *D. speciosa vigens* Erichson, *D. undecimpunctata undecimpunctata* Mannerheimer (western spotted cucumber beetle), *D. undecimpunctata howardi* Barber (southern corn rootworm), *D. virgifera zeae* Krysan & Smith (Mexican corn rootworm) and *D. viridula* (Fabricius). The key of Krysan & Miller (1986) can be used for differentiation of the adults of 13 *Diabrotica* species which occur in USA agriculture. This key (Table 1) also takes account of the distribution in North America.

Besides *D. virgifera*, larvae of *D. undecimpunctata howardi* and *D. longicornis* can feed on maize roots. Differentiation of mature larvae, alive or stored in 70% ethanol, is possible by the anal plate or pygidial shield on the ninth abdominal segment. Furthermore, the dark lines on head capsules give additional hints for the differentiation of the species (Mendoza & Peters, 1964). Urogomphi (varying considerable in size) are present in *D. undecimpunctata* (Web Figs 20 and 1b) but not in *D. virgifera* (Web Figs 20 and 2b) or *D. longicornis* (Web Figs 20 and 3b). The postventral margins of the anal plates of *D. undecimpunctata* (Web Figs 20 and 1b) and *D. virgifera* (Web Figs 20 and 2b) are dark brown in contrast to *D. longicornis* (Web Figs 20 and 3b). A typical character for the species *D. virgifera* is the darkened area with a notch on the anterior margin of the anal plate (Web Figs 20 and 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).

Table 1 Key to the adults of 13 *Diabrotica* species which occur in USA 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 disk	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 (Web Fig. 18A)
	Elytra entirely pale or with a narrow piceous vitta extending posteriorly from the humeral angle; beetle largely green	*D. virgifera zeae* (Web Fig. 18B)
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) (Web Fig. 18C)
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* (Web Fig. 18D)
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* (Web Fig. 18E)
	Spots smaller and brownish; form less robust	*D. u. tenella*

Table 2 Key to mature larvae of *Diabrotica* spp.

1	Rootworm larvae with urogomphi, head capsule side definitely rounded, and cross-like, diffuse marking on median region of frons	*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 1/3 length of frontal	*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	*D. longicornis*

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 (Web Figs 20 and 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 about one third the length of each frontal suture, creating a bifurcate pattern (Web Figs 20 and 2a). This bifurcate pattern is more diffused than the marginal band (Mendoza & Peters, 1964).

The key (Table 2) of Mendoza & Peters (1964) can be used in combination with Web Fig. 20 to differentiate mature larvae of *D. virgifera* from those of *D. undecimpunctata howardi* and *D. longicornis*.

Requirements for a positive diagnosis

The procedures for diagnosis and identification described in this protocol should have been followed. For a positive identification, adult specimens should have been studied. The specimen(s) should show the characteristics of the *Chrysomelidae*, *Galerucinae*, the genus *Diabrotica* and species *virgifera* as described here.

Report on the diagnosis

A report on the execution of the protocol should include:

- information and documentation on the origin of the infested material
- measurements and drawings or photographs (as relevant) of the morphological features required for a positive diagnosis
- the magnitude of the infestation (how many individual pests found; how much damaged tissue)
- comments as appropriate on the certainty or uncertainty of the identification
- preserved/mounted specimens may also be required.

Further information

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

Acknowledgements

This protocol was originally drafted by: P. Baufeld, Federal Biological Research Centre for Agriculture and Forestry, Kleinmachnow (DE); P. Reynaud, UFR d'Ecologie Animale et de Zoologie Agricole, Montpellier (FR).

References

Apple JW, Chiang HC, English LM, French LK, Keaster AJ, Krause GF, Mayo ZB, Munson JD, Musick GJ, Owen JC, Rasmussen EE, Sechriest RE, Tollefson JJ & Wedberg JL (1977) Impact of northern and western corn rootworm larvae on field corn. *North Central Region Research Publication* no. 239. Research Division: University of Wisconsin, Madison (US).

Baca F, Camprag D, Keresi T, Krnjajic S, Manojlovic B, Sekulic R & Sivcev I (1995) [Western corn rootworm *Diabrotica virgifera virgifera*.]. In: *Drustvo za Zastitu Bilja Srbije*. Belgrade (YU) (in Serbian).

Christensen J (1943) [Studies on the genus *Diabrotica* Chev. in Argentina.]. *Revista de la Facultad de Agronomia y Veterinaria* **10**, 464–516 (in Spanish).

Delvare G & Aberlenc HP (1989) *Les insectes d'Afrique et d'Amérique tropicale – Clés pour la reconnaissance des familles*. CIRAD/GERDAT, Montpellier (FR).

Edwards CR, Barcic J, Berberovic H, Berger HK, Festic H, Furlan L, Ivanova I, Kiss J, Princzinger G, Sivcev I & Vonica I (1999) An update on the spread of western corn rootworm in Europe. In: *6th International IWGO Workshop on Diabrotica virgifera*. Paris (FR).

EPPO/CABI (1997) *Diabrotica barberi* and *D. virgifera*. *Quarantine Pests for Europe* (2nd edn), pp. 233–237. CAB International, Wallingford (GB).

Kiss J, Khosbayar B, Komaromi J, Igrc-Barcic J, Dobrincic R, Sivcev I, Edwards CR & Hatala-Zseller I (2001) Is the western corn rootworm adapting itself to the European crop rotation system? Results of a joint European trial. In: *8th International IWGO Workshop on Diabrotica virgifera*. Venezia (IT).

Krysan JL & Miller TA (1986) *Methods for the Study of Pest Diabrotica*. Springer Verlag, New York (US).

Krysan JL, Ray FS, Branson TF & Guss PL (1980) A new subspecies of *Diabrotica virgifera*: description, distribution, and sexual compatibility. *Annals of the Entomological Society of America* **73**, 123–130.

Lawrence JF (1991) Order Coleoptera. In: *Immature Insects* (Ed. Stehr FW), 2, pp. 144–658. Kendall/Hunt, Dubuque (US).

Mendoza CE & Peters DC (1964) Species differentiation among mature larvae of *Diabrotica undecimpunctata howardi*, *D. virgifera*, and *D. longicornis*. *Journal of the Kansas Entomological Society* **37**, 123–125.

Mohr KH (1966) Chrysomelidae. In: *Die Käfer Mitteleuropas* (Ed. Freude H, Harde KW & Lohse GA), Vol. 9, pp. 95–280. Goecke & Evers, Krefeld (DE).

Petty HB, Kuhlman DE & Sechriest RE (1968) Corn yield losses correlated with rootworm larval populations. *Entomological Society of America North Central Branch Proceedings* **24**, 141–142.

Seeno T & Wilcox JA (1982) Leaf beetle genera (*Coleoptera*: *Chrysomelidae*). *Entomography* **1**, 1–221.

Wilcox JA (1972) *A Synopsis of the North American Galerucinae* (Coleoptera: Chrysomelidae). *Bulletin New York State Museum and Science Service* no. 400. Albany (US).

Fig. 1. Larvae of *D. virgifera virgifera*

Fig. 2. Damage of root pruning by larvae

Fig. 3. Adult of *D. virgifera virgifera*

Fig. 4. Adult feed on the maize silks

Fig. 5. Reduced seed set due to adult maize silk and pollen feeding

Fig. 6. Leaf damage caused by adults of Western corn rootworm

Fig. 7. Cucurbit fruit attacked by adults of *D. virgifera virgifera*

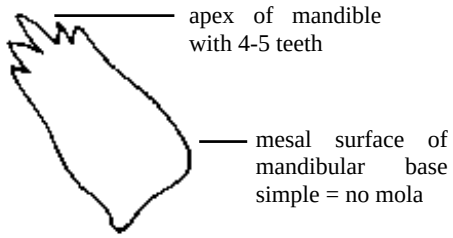

Fig. 8. Mandible

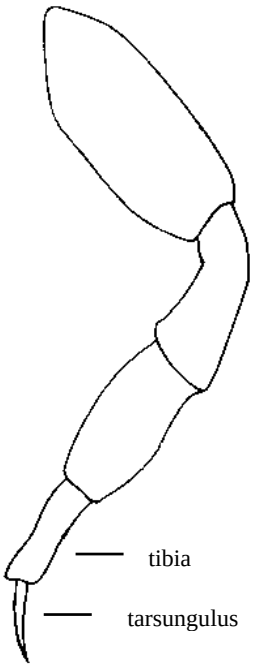

Fig. 9. Leg

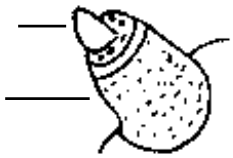

Fig. 10. Antenna

Fig. 11. Head

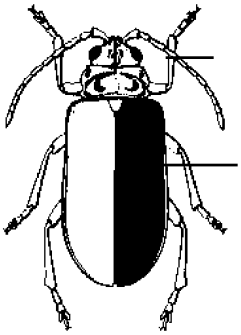

Fig. 12. General aspect of a Chrysomelidae

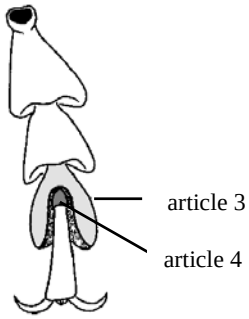

Fig. 13. Tarsus

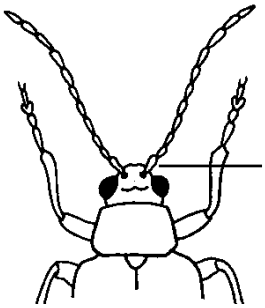

Fig. 14. Anterior part of the body

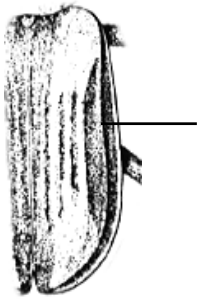

Fig. 15. Right elytra

Fig. 16. Femur

Fig. 17. Male genitalia

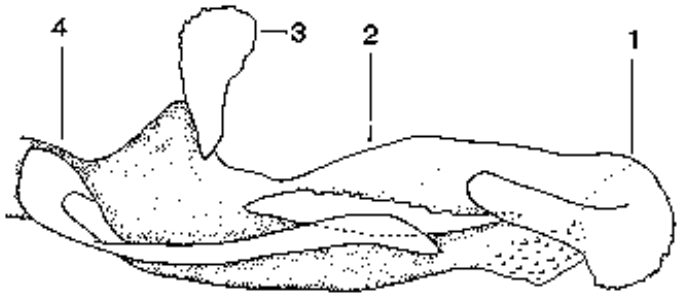

Fig. 18. Ventral view through the internal sac of aedeagus of *Diabrotica spp.*

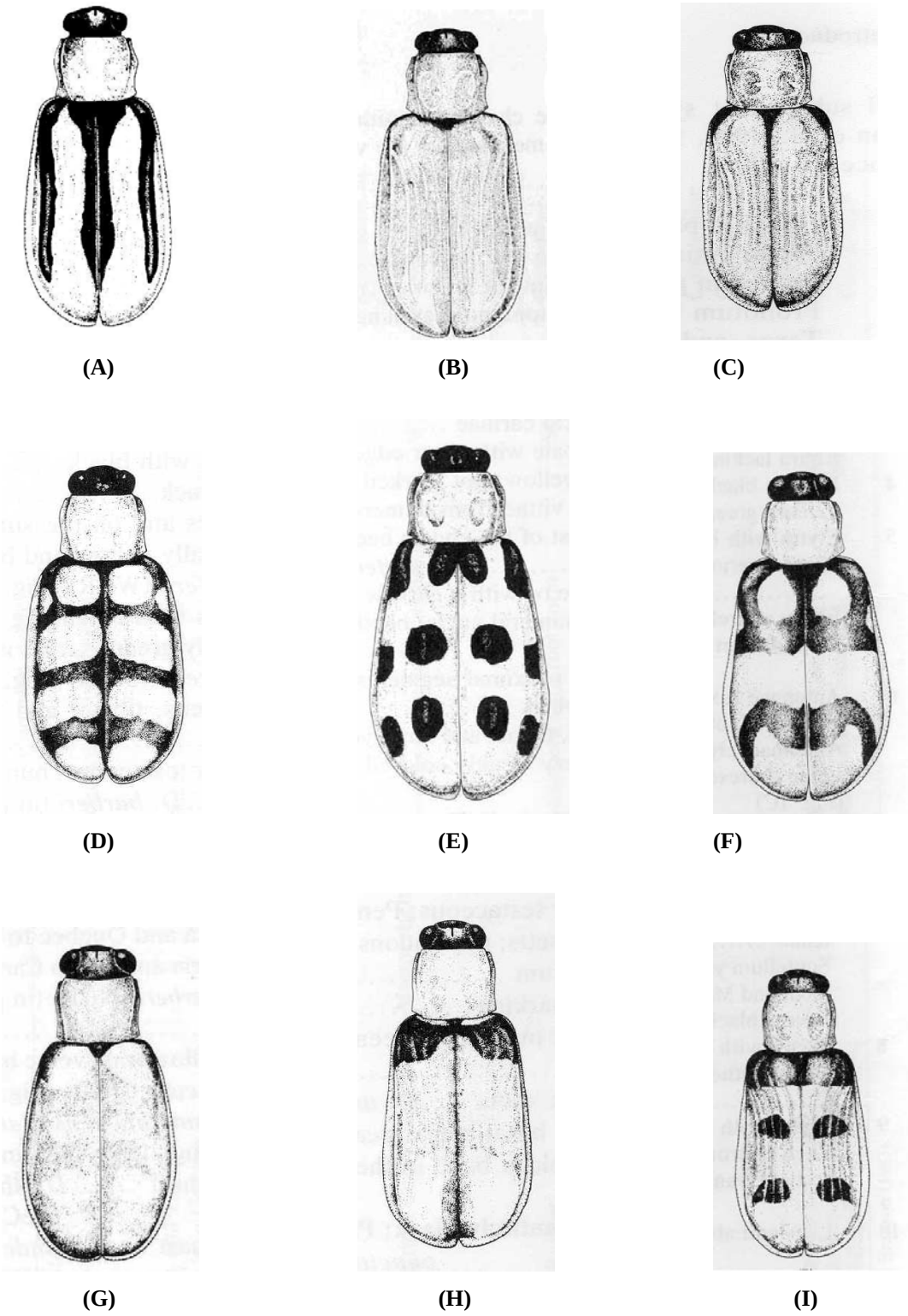

Fig. 19. **(A)** *Diabrotica virgifera virgifera* (western corn rootworm), **(B)** *D. virgifera zeae* (Mexican corn rootworm), **(C)** *D. barberi* (northern corn rootworm), **(D)** *D. balteata* (banded cucumber beetle), **(E)** *D. undecimpunctata howardi* (southern corn rootworm), **(F)** *D. adelpha*, **(G)** *D. speciosa*, **(H)** *D. viridula* (variation), **(I)** *D. viridula* (variation).

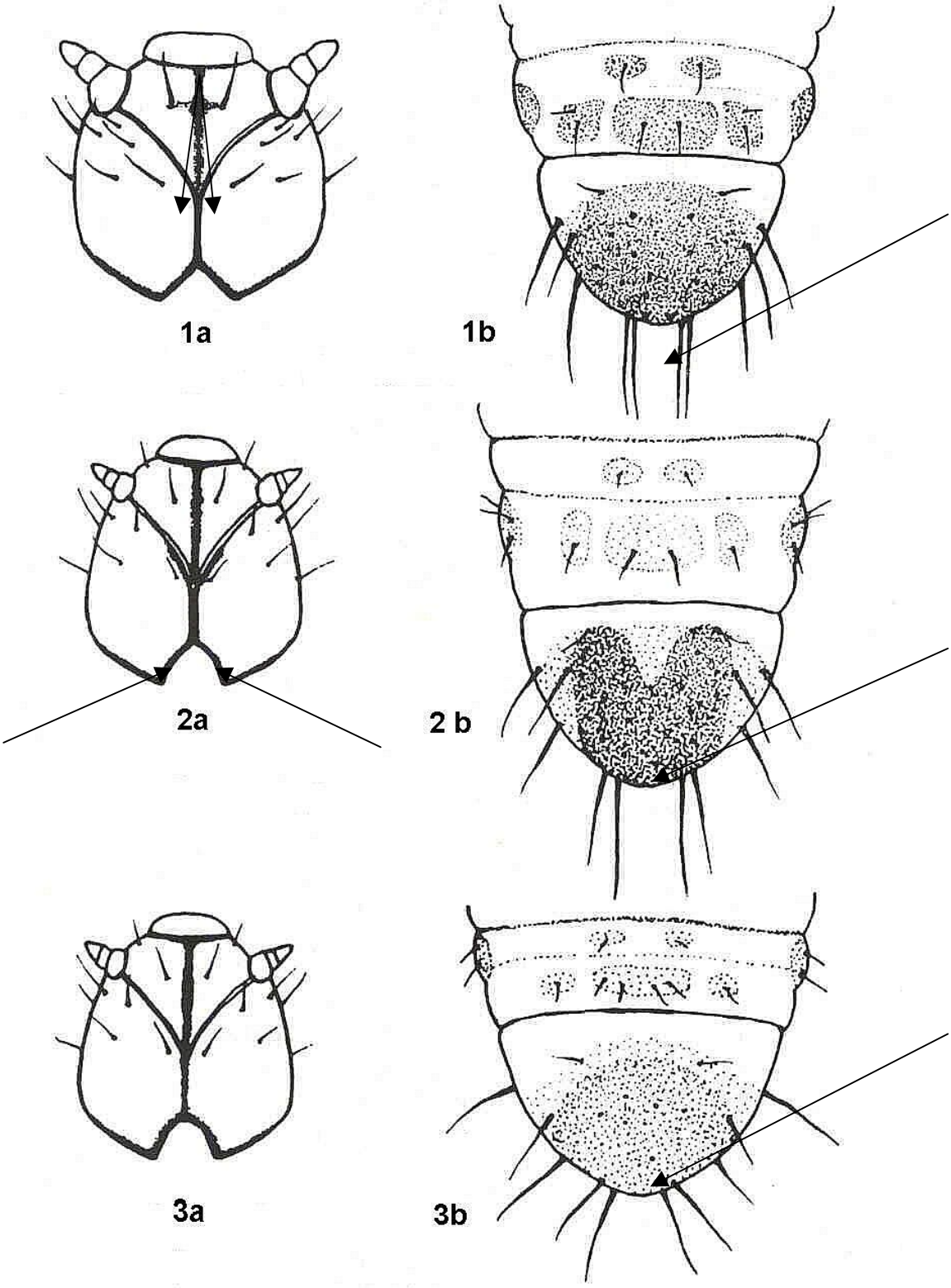

Fig. 20. Head capsule (a) and anal segments (b) of *D. undecimpunctata horwardi* (1a, 1b), *D. virgifera* (2a, 2b) and *D. longicoris* (3a, 3b).

Iconography

Fig. 1-4:

BAUFELD, P. Federal Biological Research Centre for Agriculture and Forestry, Department for National and International Plant Health, D-14532 Kleinmachnow, Stahnsdorfer Damm 81, Germany

Fig. 5:

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Fig. 6:

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Fig. 7:

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Fig. 8-11:

LAWRENCE, J.F., 1991. Order Coleoptera. pp. 144-658. In Stehr, F. W. (ed.) *Immature Insects*. Kendall/Hunt, Dubuque, Iowa. Vol. 2. xvi + 975 pp.

Fig. 12:

LAWRENCE, J.F., BRITTON, E.B.,1991. Coleoptera. pp. 543-683. In *The Insects of Australia : a Textbook for Students and Research Workers.* (CSIRO ed.) Cornell University Press, Vol. II. 1137 pp.

Fig. 13:

DELVARE, G., ABERLENC, H.P., 1989. Les insectes d'Afrique et d'Amérique tropicale – Clés pour la reconnaissance des familles. *CIRAD/GERDAT*, Montpellier, 302 pp.

Fig. 14:

MOHR, K.H., 1966. Chrysomelidae. pp. 95-280 in H. Freude, K.W. Harde & G.A. Lohse (Eds.), *Die Käfer Mitteleuropas*. Gaecke & Evens, Krefeld. Vol. 9.

Fig. 15-18:

KRYSAN, J.L., RAY, F.S., Branson, T.F., Guss, P.L., 1980. A new Subspecies of *Diabrotica virgifera* (Coleoptera: Chrysomelidae): Description, Distribution, and Sexual Compatibility. *Annals of the Entomological Society of America* **73**, 123-130.

Fig. 19:

KRYSAN, J.L. & MILLER, T.A., eds., 1986. Methods for the study of Pests Diabrotica. *Springer-Verlag*, New York, 260 pp.

Fig. 20:

MENDOZA CE & PETERS DC (1964) Species differentiation among mature larvae of *Diabrotica undecimpunctata horwardi*, *D. virgifera*, and *D. longicornis*. Journal of the Kansas Entomological Society **37**, 123-125.