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SAPERDA TRIDENTATA OLIVIER (COLEOPTERA CERAMBYCIDAE LAMIINAE): CONTINUOUS INTERCEPTIONS AT THE ITALIAN PORT OF LIVORNO REPRESENT A GROWING CHALLENGE FOR PHYTOSANITARY SERVICES

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Binazzi F., Del Nista D., Sabbatini Peverieri G., Marianelli L., Roversi P.F., Pennacchio F. – *Saperda tridentata* Olivier (Coleoptera Cerambycidae Lamiinae): continuous interceptions at the Italian port of Livorno represent a growing challenge for phytosanitary services.

Trade of plant material poses a potential risk for accidental introductions of alien pests. Despite prevention and control measures, many exotic species have been unintentionally introduced into the Italian peninsula in the last decades. From April 2016 to March 2019, infested wood material from several areas of the USA was intercepted by phytosanitary authorities at the port of Livorno (Tuscany, Italy). The longhorn beetle *Saperda tridentata* was detected in six shipments containing heavily infested elm logs (*Ulmus rubra*) with bark. All the interceptions were made thanks to a national monitoring network including the Italian Ministry of Agriculture and Forests (MIPAAF), the Regional Phytosanitary Services, the Research Centre for Plant Protection and Certification (CREA-DC) and other Italian scientific institutions. Our findings suggest that monitoring of timber shipments should be further developed not only at the entry ports but also, and particularly, in the countries of origin. Moreover, a shift in phytosanitary policies such as introducing mandatory wood treatments (e.g. debarking, fumigation or heat treatments) prior to shipment would be advisable as it could drastically reduce introductions of alien pests and streamline all inspection procedures.

KEY WORDS: exotic species, timber trade, monitoring activities, pest risk assessment, alien species.

INTRODUCTION

Timber trade and the widespread use of wood as packaging material represent a potential risk for accidental introductions of xylophagous beetles belonging mainly to the families Cerambycidae, Buprestidae and Curculionidae (CAVEY, 1998; HAACK, 2001, 2006; HAACK *et al.*, 2010; LIEBHOLD *et al.*, 1995; MCCULLOUGH *et al.*, 2006; AUKEMA *et al.*, 2010, HAACK & RABAGLIA, 2013; PENNACCHIO *et al.*, 2003, 2004, 2012a, 2012b, 2016; RASSATI *et al.*, 2017; STRANGI *et al.*, 2017; MEURISSE *et al.*, 2019; TORRINI *et al.*, 2019).

In the last decades, despite prevention and control measures, many alien species have been unintentionally introduced into the Italian peninsula. Some of them often spread and established in new territories with negative consequences for agriculture and biodiversity conservation (INGHILESI *et al.*, 2013, PENNACCHIO *et al.*, 2012a; HERARD *et al.*, 2009; BULLAS-APPLETON *et al.*, 2014; TORRINI *et al.*, 2016; MAZZA *et al.*, 2017). Nonetheless, national control networks recently established in high-risk areas such as airports, customs, ports and nurseries allowed the detection of hundreds of xylophagous species, belonging mainly to the families Cerambycidae, Buprestidae, Bostrichidae and Curculionidae (particularly Scolytinae). In particular, thanks to the monitoring activities implemented at the Italian port of Livorno, several interceptions of infested wood material were made in recent years. Among them, the longhorn beetle *Saperda tridentata* Olivier (Cerambycidae, Lamiinae) has been continuously recorded in elm wood shipped from

the USA since the spring of 2016. The adults of this woodborer feed on elm leaves and twigs while larvae colonize trunks forming extensive galleries under tree bark. In all inspections, logs with bark resulted heavily colonized by mature larvae of *S. tridentata* often associated with bark beetles (Scolytinae) at various development stages.

MATERIALS AND METHODS

Inspection methods performed by phytosanitary services may vary according to context and type of shipped material. During the inspections of elm wood the following standard procedure was carried out: Metal containers were opened at the port in special facilities and phytosanitary authorities examined the entire content. Since insects are attracted to light, particular attention was paid to the potential presence of live adults able to escape through the openings. If free-walking insects or any flying activity were observed, containers were immediately closed and all wood material subjected to fumigation and/or thermal treatments before performing further investigations. If no potentially dangerous insect activity was detected, all items including packaging material and/or plastic envelop or other synthetic elements used to wrap wood pallets were screened for the presence of live organisms. Pavement and all the inner frame of containers were also carefully inspected.

All inspections were carried out using specific cutting tools (e.g. hatchets, knives, gouges, chisels), inspection

tools (e.g. magnifying glasses, mirrors, electric torches) and other electronic devices such as portable field USB-microscopes. All live organisms and more generally all suspected material including wood pieces, sawdust, frass and any type of debris were collected from logs and/or wood items and placed into tubes or metal containers for further laboratory evaluations. Moreover, all debris including earth, sand, sawdust and frass scattered on the container pavement was equally collected, stored with the same procedure and finally screened in the laboratory for potential biological material. When logs with bark were inspected, a special focus was placed on detecting holes and insect galleries. In fact, their presence may indicate infestations due to bark beetles, ambrosia beetles and other woodborers. Moreover, when holes and galleries on the inner wood appeared dark and/or clearly surrounded by abundant darkish frass, particular care was taken in the inspections. In fact these characteristics might be effective indicators of the presence of insect-associated phytoparasitic nematodes or fungi such as *Ambrosia* spp. (Fig. I, 1, 2, 3).

All collected material was then placed into robust plastic bags and stored in climatized rooms or refrigerators. Subsequently, material was observed in the laboratory with a stereomicroscope equipped with a cold light source. Bark and wood material were thoroughly inspected to detect the

presence of insect larvae, pupae and adult stages. When insects were found, specimens were morphologically identified with keys and/or stored in 96% ethanol for further molecular evaluation (PENNACCHIO *et al.*, 2012b; SABBATINI PEVERIERI G., 2012). All waste material useless for the identification was autoclaved and then discarded.

RESULTS

From April 2016 to March 2019, the abovementioned procedures allowed phytosanitary authorities at the port of Livorno (Tuscany, Italy) to inspect six shipments containing wood material from several areas of the USA. Once containers were opened, no flying activity was detected but several logs of *Ulmus rubra* presented on the outer bark clear symptoms of insect infestations such as penetration holes and/or frass due to intense activity of xylophagous species.

Subsequent wood debarking evidenced the presence of insect pupae and larvae at various development stages, with particular abundance of *S. tridentata* mature larvae. In the same infested logs, larvae, pupae and adults of the scolytid *Scolytus multistriatus* Marsham coexisting with *S. tridentata* were also observed (Tab. 1) (EUROPHYT, 2019). Pupae and several mature larvae of both *S. tridentata* and

Fig. I – 1. Detail of a *Saperda tridentata* mature larva; 2. Adult male of *Saperda tridentata*. 3. Inspection procedures of *Ulmus rubra* logs shipped in metal containers.

Table 1 – Shipments of *Ulmus rubra* logs infested with *Saperda tridentata* and *Scolytus multistriatus*.

Interception Dates	Country of origin	Quant. (m ³)	Intercepted Species	Development Stages
04/08/2016	USA, Ohio	3,8	<i>Saperda tridentata</i>	Larvae at various development stages
01/02/2017	USA, Virginia	7,8	<i>Saperda tridentata</i>	Larvae at various development stages
08/05/2017	USA, Illinois	18,2	<i>Saperda tridentata</i>	Larvae at various development stages
11/10/2017	USA, Iowa	18,3	<i>Saperda tridentata</i>	Larvae at various development stages
23/10/2018	USA, Ohio	18,8	<i>Saperda tridentata</i> , <i>Scolytus multistriatus</i>	<i>S. tridentata</i> larvae at various development stages; larvae and adults of <i>S. multistriatus</i>
27/03/2019	USA, Iowa	19,6	<i>Saperda tridentata</i> , <i>Scolytus multistriatus</i>	<i>S. tridentata</i> larvae at various development stages; larvae and adults of <i>S. multistriatus</i>

S. multistriatus were reared in climatic chambers set at 26 ± 2 °C and 75 % RH until adult emergence.

BIOLOGY

The first account on the biology of the Lamiid, *S. tridentata* on elm in southeastern New York dates back to the first half of the 20th century (PECHUMAN, 1940).

As the vast majority of saperdini, *S. tridentata* has an annual life cycle in temperate climates. Adult males are blackish fading to reddish, subparallel and 10-14 mm long, with antennae almost as long as the body. A dense grey pubescence with three orange oblique crossbars covers the elytra while narrow stripes are visible on each side of pronotum and base of the elytra. Adult females are similar to males but they are more robust and slightly longer (13 to 17 mm) (Fig. 1, 2). Adult emergence starts at the beginning of the spring (early May) and continues until the mid of July, with peaks in the last week of May and in the first of June. This pest has an unbiased sex ratio with males emerging earlier than females (PECHUMAN, 1940; LINSLEY & CHEMSAK, 1995; EPP0, 2019). Adults are active mainly at night while during the day they rest on both tree bark and foliage. Mating occurs one or two weeks after the emergence and females, who can live up to 1 month in the field, start oviposition only after feeding on leaves and twigs. Each female can lay up to 50-60 eggs, which are deposited singly into incisions made with mandibles in bark crevices (HOFFMANN, 1939; PECHUMAN, 1940).

After hatching, larvae penetrate into the phloem where they form extensive feeding galleries. In already weakened plants larvae may migrate from already colonized parts to healthy ones often causing death of the upper part of tree canopies. Mature larvae are white-yellowish, limbless and approximately 30 mm long even though their size may vary considerably depending on food sources and other environmental factors (Fig. 1, 1). In fresh wood, larvae are generally active in the space between the inner bark and the external layer of the sapwood. From August to October, they start to dig chambers for overwintering and at the end of the winter they penetrate deeper to form pupal cells (PECHUMAN, 1940; EPP0, 2019). These structures are usually constructed 5-6 mm below the sapwood surface or in thick bark and are plugged with frass. Pupal stage develops typically between March and April and may last from 15 to 33 days (PECHUMAN, 1940; EPP0, 2019). Newly formed adults often remain in the pupal cells for about a week before emerging through the larval tunnel or digging their way out. Emergence is generally affected by temperature and insects tend to follow the shortest route for

reaching the surface. However, in case of sawed wood, adults have been sometimes observed to emerge at the end of logs depending on the position of pupal chambers.

In its native range, the life cycle of *S. tridentata* is highly variable even among individuals originating from the same group of eggs. The entire development period is usually one year but in some circumstances it was reported to be up to more than three years depending on temperature and moisture content in the wood (HOFFMANN, 1939; PECHUMAN, 1940; EPP0, 2019).

DISTRIBUTION

The genus *Saperda* Fabricius, 1775 includes the following 8 species in continental Europe: *Saperda carcharias* (L., 1758), *S. similis* Laicharting, 1784, *S. punctata* (L., 1767), *S. populnea* (L., 1758), *S. scalaris* (L., 1758), *S. octopunctata* (Scopoli, 1772), *S. perforata* (Pallas, 1773), *S. quercus* Charpentier, 1825. All of them have been recorded in Italy except for *S. quercus*.

Conversely, *S. tridentata* is native to central Eastern North America and its range is wide, extending approximately from southeastern Canada, to Texas (SALOMON, 1995; BOBERG & BJÖRKLUND, 2018). In particular, records have been reported from the following North American states: in Canada (Manitoba, New Brunswick, Nova Scotia, Ontario, Québec, Saskatchewan) and in the USA (Alabama, Arkansas, Colorado, Connecticut, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, West Virginia, Wisconsin) (EPP0, 2019). It is worth noting that this species has been frequently observed in the northeastern states of United States (e.g. New York and Massachusetts) and that no records have been reported outside its native range so far (SALOMON, 1995; BOBERG & BJÖRKLUND, 2018; EPP0, 2019).

HOST PLANTS AND POTENTIAL RISKS

Elms are emblematic and valuable trees highly prized for landscaping and wood production in all the EPP0 countries. Regrettably, in the last decades, these plants have been gradually decimated by epidemics of Dutch elm disease (i.e. *Ophiostoma ulmi*, *O. novo-ulmi*) primarily vectored by *Scolytus* spp.

S. tridentata is associated with several North American *Ulmus* species. Its main host is *U. americana*, but infestations have been also observed on *U. rubra* and *U. crassifolia*. To date, there is very limited knowledge on the potential susceptibility of all the other North American elms (KRISCHIK & DAVIDSON, 2013). Equally controversial is the possible vulnerability of Palearctic elms located in the EPPO regions. Among the European elms, there is particular concern for the native *U. glabra*, *U. laevis*, *U. minor* and the introduced *U. pumila* (BOBERG & BJÖRKLUND, 2018). The latter one, native to East Asia, is resistant to Dutch elm disease and has been widely used for landscaping in countryside areas or as a street tree in urban environments.

S. tridentata, though normally regarded as a secondary pest of weakened trees in North America, may become lethal for elms in case of high infestation levels (FELT & JOUTEL, 1904; TUCKER, 1907; HOFFMANN, 1939; PECHUMAN, 1940; EPPO, 2019). Recently-cut logs and weakened trees suffering from root injury, diseases, water deficiency or other stress factors are usually the first choice for ovipositing females even though attacks have been sometimes reported also on apparently healthy plants (HOFFMANN, 1939; PECHUMAN, 1940).

The first symptom of *S. tridentata* attack in apparently vigorous trees is the appearance of a generalized yellowing and thinning of foliage followed by dieback and death of some branches (often scattered over the tree). At the beginning of insect colonization, reddish frass extruded from tiny openings in bark crevices, can be observed on either tree trunks or branches. As the infestation progresses, bark tends to lose adherence to wood until large pieces can be easily stripped from the main trunk and widespread larval galleries can be detected in the inner bark (PECHUMAN, 1940; EPPO, 2019). During the peak of infestation, larvae boring galleries may gradually girdle elm branches or trunks ultimately causing tree death (HOFFMANN, 1939; PECHUMAN, 1940).

Unlike other longhorn beetles such as *Trichoferus* spp. or *Clytus* spp., which preferentially exploit dehydrated material and repeatedly colonize the same wood portions for more generations, *S. tridentata* is more attracted to fresh non-colonized areas of already attacked trees. Since weakened but still fully hydrated elms are so attractive, larvae of *S. tridentata* and occasionally *Scolytus multistriatus* rapidly exploit the suitable wood depleting all the available food sources.

Elms already declining due to infestations of *Ophiostoma ulmi* or *O. novo-ulmi* are particularly exposed to *S. tridentata* attack and may be gradually colonized by this pest as Dutch Elm disease progresses. Furthermore, since *S. tridentata* has been also reported as a vector of *Ophiostoma ulmi*, European elms might be threatened not only directly by this longhorn beetle but also indirectly by vector-borne infections (EPPO, 2019). This scenario represents thus a further matter of concern, as this beetle, once introduced into Europe, might not only extensively damage elms already weakened by Dutch elm disease but also contribute to the spread of the disease itself. Therefore, in 2019 this beetle was included in the EPPO alert list.

PHYTOSANITARY MEASURES AND POLICIES

Biological invasions have been often reported to have negative impacts on newly colonized environments with important phytosanitary implications. In some cases,

though, damages to crop production and natural ecosystems due to accidentally introduced species could be theoretically predicted based on previous knowledge on their biology, behaviour and ecology in the native ranges. Check lists of organisms potentially harmful to newly invaded ecosystems can be generated in order to improve long-term monitoring of high-risk areas particularly exposed to possible introduction of dangerous species such as alien phytophagous insects (Annex I Part A, of the COUNCIL DIRECTIVE 2000/29/EC, lists A1, A2 e Alert list EPPO).

It should be stressed anyway that it is often complex to evaluate a priori the potential threat posed by invasive organisms in new environments (SABBATINI PEVERIERI *et al.*, 2017). In fact, there is growing evidence that even species apparently harmless in their native range might turn into more aggressive pests once established in new ecosystems (EL KHOURY *et al.*, 2019).

In the last decades, the accidental introduction of several alien xylophagous insects evidenced the potentially negative impact of global trade in logs, woody plants, packaging material and wood pallets on newly colonized environments. Among the alien organisms recently introduced into Italy, *Anoplophora chinensis* Thomson, *A. glabripennis* (Motschulsky), *Aromia bungii* (Faldermann), *Psacotha hilaris* (Pascoe) (Coleoptera Cerambycidae), *Xylosandrus germanus* (Blandford), *X. crassiusculus* Motschulsky, *X. compactus* (Eichhoff), *Phloeotribus liminaris* (Harris), *Pityophthorus juglandis* Blackman, *Ambrosiodmus rubricollis* (Eichhoff), *Xyleborus atratus* Eichhoff, *Hypocryphalus scabricollis* (Eichhoff) (Coleoptera Curculionidae Scolytinae), *Megaplatypus mutatus* (Chapuis) (Coleoptera Curculionidae Platypodinae) and *Aclees* sp. (Coleoptera Curculionidae) deserve particular attention in terms of phytosanitary risk. Equally noteworthy for their potential phytosanitary implications are the interceptions of *Phryneta leprosa* (Fabricius) and *Coptops aedificator* (Fabricius) (Coleoptera, Cerambycidae Lamiinae) under the bark of *Milicia excelsa* (Welw.) C.C.Berg (Iroko) trunks shipped from Cameroon and the interceptions of *Tricoferus campestris* (Faldermann) captured by traps located in the port of Napoli and likely vectored through packaging material (PENNACCHIO *et al.*, 2003; PENNACCHIO *et al.*, 2004; SABBATINI PEVERIERI *et al.*, 2012a; PENNACCHIO *et al.*, 2012b; PENNACCHIO *et al.*, 2016; STRANGI *et al.*, 2017; FRANCARDI *et al.*, 2017).

Several of the abovementioned species resulted harmful in newly colonized areas, even though they had never been officially listed as noxious organisms before their introductions (e.g. *Xylosandrus crassiusculus*, *X. compactus*, *Pityophthorus juglandis*) or before their repeated interceptions (e.g. *Saperda tridentata*). Likewise, some species evidently harmful to *Ficus carica* such as *Aclees* sp. *Psacotha hilaris* and *Hypocryphalus scabricollis*, have never been included in any phytosanitary alert list so far.

These records suggest that trade of wood with bark represents a potential risk factor for all EU countries. Recent literature further highlights that Mediterranean areas are particularly exposed to potential introduction and establishment of alien pests native to temperate but also tropical and subtropical regions (RASSATI *et al.*, 2016; EL KHOURY *et al.*, 2019). Moreover, Italy for its peculiar geographical location and extreme variety of habitats (e.g. mountain environments, plains, coastal areas) is probably the Mediterranean state most at risk to accidental pest introduction, dispersal and establishment (PENNACCHIO *et al.*, 2016).

The frequent interceptions of *S. tridentata* are in line with the trend described above. In fact the main vectors of this pest are either infested wood traded with bark or packaging material that may host larvae, pupae and adults. In particular, shipments of recently cut fresh wood represent a highly efficient network of pest transfer and should deserve special care from phytosanitary authorities (PENNACCHIO *et al.*, 2016).

In its native range, *S. tridentata* is rarely a matter of concern and phytosanitary measures against this beetle are focused mainly on silvicultural practices aimed at maintaining trees vigorous such as removal and burning of severely infested trees to decrease insect populations (HOFFMANN, 1939).

On the other hand, in Europe, regular trade of infested elms and the capacity of *S. tridentata* to transmit Dutch elm disease might represent a risk in case this beetle should be introduced into Italy. Nonetheless, there is still controversy on the actual susceptibility of elm species in the EPPO regions, whereas a general lack of information prevents a thorough evaluation of the economic impact of this pest even in its native range (EPPO, 2019).

Field experience and scientific literature suggest, though, that once alien species are established in a new ecosystem their complete eradication is often complex. Therefore, ideal phytosanitary measures should require accurate wood inspections aimed at diagnosing infestations due to xylophagous insects (e.g. presence of galleries and frass) or other dangerous organisms and/or diseases. Positive diagnosis should be then followed by complete wood debarking and/or other phytosanitary measures such thermal treatments or fumigation. Even more important, all inspections, diagnosis and eventual treatments should be performed in the countries of origin before shipments take place. That would not only benefit trade reducing the risk of infested material being refused at the entry ports, but would also protect the entire community and environment from potentially dangerous biological invasions (PENNACCHIO *et al.*, 2012b, 2016).

Regrettably, according to the current regulations, most of broadleaved woody species shipped from North America, tropical and subtropical Asia, Africa and South America are not subjected to any debarking procedure nor to any standard phytosanitary treatment or compulsory inspections by phytosanitary authorities.

In accordance with PENNACCHIO *et al.*, 2016 and POLAND & RASSATI, 2018, our findings underline the importance of implementing the active monitoring of areas highly exposed to accidental introductions of alien species and to improve the alert system designed to limit the spread of exotic pests in the Italian territory.

The abovementioned interceptions of *S. tridentata* were carried out thanks to a national monitoring network including MIPAAF, regional phytosanitary services, CREA and other Italian scientific institutions. Nonetheless, we believe that both prevention and monitoring activities should be further developed not only at the entry ports but also, and particularly, in the countries of origin. Moreover, we emphasize the importance of improving the compliance with the current international legislation concerning safety measures for packaging material, in agreement with the FAO requirements (ISPM No. 15) (2013). At the same time, a shift in phytosanitary policies should be highly recommended, as field experience suggests that introducing mandatory wood debarking prior to shipment could drastically reduce introductions of alien pests and effectively streamline all inspection procedures.

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