A review of pest surveillance techniques for detecting quarantine pests in Europe

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A review of pest surveillance techniques for detecting quarantine pests in Europe*

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This paper provides reviews of the most commonly used methods to detect plant pests belonging to groups of invasive organisms with high economic relevance, including Coleoptera (bark beetles, flathead borers, leaf beetles, longhorn beetles, weevils), Diptera (cone and seed flies, fruit flies), Homoptera (aphids, leafhoppers and psyllids, whiteflies), Lepidoptera (moths and butterflies), Thysanoptera (thrips), bacteria (potato brown rot *Ralstonia solanacearum*) and fungi (pitch canker disease *Gibberella circinata*, brown rot disease *Monilinia fructicola*). Future perspectives in detection methods are discussed, with particular reference to the considerable increase in the volume, commodity type and origins of trade in plant material from third countries, the introduction of new crops, the continuous expansion of the EU with new border countries being added, and the impact of climate change affecting the geographical boundaries of pests and their vectors.

Introduction

The threats posed by new plant pests are now greater than ever (Baker *et al.*, 2005). The main reasons are (a) considerable increases in the volumes, commodity types and origins of trade in plant material from third countries; (b) the introduction of new crops; (c) the continued expansion of the EU, with new border countries being added; and (d) the impact of climate change affecting the distributions of pests and their vectors. Although there are no published figures estimating the cost of 'all' non-native pests for the 'whole' of Europe, there are some estimates for certain pests and certain countries that indicate a great economic loss caused by exotic pests (Vilà *et al*., 2009). According to Baufeld & Enzian (2005), the introduction of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte; Coleoptera: Chrysomelidae) has cost Europe around 147 million EUR per year. For the UK, Pimentel (2002) estimated that invading insect pests and plant pathogens cause 6.3 billion EUR damage to crops and forests annually. As a result, UK government departments contribute about 18 million EUR per year to quarantine plant health activity, mostly in the area of risk reduction. According to Kenis & Branco (2010), a similar calculation for the entire EU would lead to annual economic losses of approximately 10 billion EUR caused by alien arthropods, not including control, eradication or quarantine costs, or costs linked to foreign trade impact of market effects.

Looking further afield, in the USA the cost of non-native pests and diseases and their control is estimated to be 95 billion EUR (Pimentel *et al.*, 2005) and invasive alien species may cause over 249 billion EUR per year worldwide in damage and control costs (Pimentel, 2002). In the USA, the Animal and Plant Health Inspection Service (APHIS) increased its annual spending on emergency eradication programs more than twenty-fold during the 1990s, from 8.2 million EUR in 1990 to 184 million EUR in 2000. In a recent study, the number of plant pests establishing in Europe has been predicted to increase significantly in the next 10 years based on current trends (EU project DAISIE at www.europe-aliens.org; Roques, 2011, Sache *et al.*, 2011). Such organisms cause considerable economic and societal damage within agriculture, horticulture, forestry and natural ecosystems. This has been amply demonstrated by the impacts of a range of different exotic plant pests such as the bacterial pathogens *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum*; the sudden oak death pathogen *Phytophthora ramorum*; Pepino mosaic

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virus; the pine wood nematode *Bursaphelenchus xylophilus*; the western corn rootworm *Diabrotica virgifera virgifera*; and the Asian and citrus longhorn beetles *Anoplophora* spp.

Monitoring such pest damage requires solid knowledge of a vast range of sometimes only subtly different symptoms and is thus highly error-prone. Furthermore, existing phytosanitary monitoring procedures may not allow the detection of pest infestation at a low level or during latent infections. This is critical as undetected infestations can result in the introduction of pests. Furthermore, recent EU mandates under Directive 2000/29 to test for latent infestations currently lack validated detection/surveillance protocols for many pests. According to International Standard for Phytosanitary Measures (ISPM) No. 6, all actions should be taken under the policy of 'Good Surveillance Practice'. These Standards require adequate training for personnel involved in phytosanitary surveillance, identification, sampling methodology, data management, record-keeping, and preservation and transportation of samples.

Surveillance is defined as 'an official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures' by the International Plant Protection Convention (IPPC), ISPM No. 5. The IPPC has also adopted 'Guidelines for surveillance' (ISPM No. 6, 2006) which aim to support National Plant Protection Organizations' (NPPO) declarations of pest freedom, aiding early detection of new pests, and compiling host and commodity pest lists and distribution records.

Surveillance techniques for exotic insect pest detection have been reviewed recently with special attention to attractants and trapping systems (Quilici *et al.*, 2012). Commonly used methods of surveillance for 177 arthropod species included in the quarantine lists of EU (Directive 2000/29 and the European and Mediterranean Plant Protection Organization, EPPO) are described in Augustin *et al.* (2012).

In this paper, the above work is expanded to the main groups of quarantine pests by organizing the information into sections including:

(a) general information on the pest in relation to surveillance;
(b) detection and monitoring methods;
(c) pathways and commodities relevant to surveillance.

Because of the different nature of the organisms involved (arthropods and microorganisms), the authors followed a different approach. For arthropods, this paper provides examples for the most important groups and species in each taxonomic category. For microorganisms, a few examples of species representing large groups are given.

The critical analysis of surveillance methods was carried out taking into account best practice criteria based on existing knowledge. Unfortunately, for some groups the information available is still too scarce to identify the best methods to choose for the surveillance of quarantine species. In the final part of this review, on future perspectives, recommendations to improve the surveillance of quarantine organisms are given.

Surveillance techniques for exotic pests

Bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae)

(M. Faccoli)

Introduction

Bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) include about 6000 species known worldwide, with the main centre of species diversity in the tropical and subtropical regions. About 500 species occur in North America and 900 in the Palaearctic region, of which about 350 occur in Europe. Scolytids are among the most common wood-boring insects transported inside wooden products and wood packaging materials (Haack, 2001; Brockerhoff *et al.*, 2006; Knížek, 2007). About 50 species are included in the different lists of harmful organisms (quarantine lists, alert lists, national or regional lists). Invasive scolytids pose a major threat to forest resources around the world (Marini *et al.*, 2011). Although the ecological and economic effects of many immigrant species are minor, some alien species can have significant impacts on the functional properties of ecosystems, disrupt food webs and displace indigenous species (Kenis *et al.*, 2009).

With respect to exotic wood-boring insects, there are effective surveillance networks in New Zealand, Australia, Canada and the USA, but the situation in Europe, Asia, Africa and South America is not as satisfactory (Knížek, 2007). For North America, a great deal is known about which invasive species are present and where they occur (Haack, 2001, 2006; Haack & Rabaglia, 2011), whereas in Europe much less is known about the numbers and distributions of alien species (Kirkendall & Faccoli, 2010; Marini *et al.*, 2011). The successful establishment of exotics appears to be accelerating despite greater international awareness of the dangers posed by wood packaging materials (FAO, 2002) and stricter regulation of plant trade. The establishment rate in Europe of new alien species of scolytids has increased markedly in the past 30 years (Faccoli, 2008, 2010; Faccoli *et al.*, 2009; Kirkendall & Faccoli, 2010; Sauvard *et al.*, 2010; Faccoli *et al.*, 2012), and a carefully targeted monitoring scheme is required.

Bark and ambrosia beetles are known to be often associated with phytopathogenic fungi (Kirisits, 2004). Serious forest diseases occurred following the introduction of bark beetles into new territories, such as *Scolytus multistriatus*, a vector of the fungal pathogen causing Dutch elm disease (Webber, 2000). In Northern Spain, 11 bark beetles species were shown to be associated with *Fusarium circinatum* transmission. Bioassays using funnel traps baited with verbenone were performed to test a possible IPM strategy (Romon *et al.*, 2007). Fox *et al.* (1991) demonstrated that *Ips mexicanus* and *Ips paraconfusus* could transmit pitch canker disease to *Pinus radiata*, and that when these species are associated with *Fusarium circinatum*-infected Monterey

pines in California, this may indicate a higher risk of pitch canker transmission depending on the propagule load they show in spring (Erbilgin *et al.*, 2008). The twig bark beetles *Pityophthorus setosus* and *Pityophthorus carmeli* are known as *Fusarium circinatum* vectors in California, where wounded healthy branches become suitable for infection (Sakamoto, 2007; Sakamoto *et al.*, 2007). Erbilgin *et al.* (2009) suggested that an initial infection by these beetles may induce resistance to subsequent infections of the host. *Conophthorus radiatae* and *Pityophthorus* spp., which infest *Pinus radiata*, are another potential source of inoculum for *Fusarium circinatum* (Hoover *et al.*, 1995, 1996).

Detection and monitoring

Trapping systems

The early detection of alien scolytid species may be achieved in two ways: trapping of flying adults or sampling of infested materials. The small size of the scolytids, the difficulty in species identification (especially of the larval instars), the relatively rapid development time, the quick emergence of the new adults, and the possible colonization of both sapwood (by *Ambrosia* beetles) and bark (by bark beetles) make scolytids very hard to detect and identify by sampling woody articles (merchandise). However, scolytids are easily trapped using specific pheromone and attractant lures. As in many other insect families, pheromones and attractive lures, when available, have two main practical applications in bark beetle management: survey (and monitoring) and mass trapping. Mass trapping may have important applications for the management of outbreak populations, including invasive alien species established in a new area. Specific aggregation pheromones may be extremely useful in the early detection and monitoring of quarantine species. The use of pheromone traps in high-risk areas, such as harbours and airports, may give information useful in early detection, quarantine operations, timing of control measures and monitoring of possible insect dispersal. However, in Europe there is no coordinated pheromone-based programme for detection and monitoring of quarantine bark and ambrosia beetles. In recent years, some European countries such as Italy, France and the UK (unpublished data), have attempted to build a regional network for the detection and monitoring of alien scolytids. However, these attempts have not been coordinated and the methodologies and tools varied among countries.

Lures

Most bark and ambrosia beetles release aggregation pheromones attracting both males and females (Byers, 2004). The pioneer adults find a suitable host-tree by following tree volatiles such as monoterpenes, sesquiterpenes and alcohols. At the beginning of host colonization, the pioneers (males or females according to the species) release the aggregation pheromones to increase the infestation density on the host-tree and, in this way, overcome the tree's defences (Byers, 2004). These lures, i.e. specific aggregation pheromones and generic attractants (host volatiles), are widely applied in IPM programmes against scolytids (Byers, 2004). Although specific semiochemicals are known for many species, only the pheromones of the most damaging American and European species are produced at an industrial scale and are available commercially. For Asian, African and tropical bark beetles, there is a lack of specific research and scientific publications on the chemical ecology of even the most common species. For these species, there are no specific lures available and only generic attractants can be used in early detection programmes.

Traps

Various trap types have been developed in the past and a few are currently widely used worldwide in detection and monitoring protocols. Aggregation pheromones are generally used to bait funnel traps, windows traps and cross-vane traps. The same trap model may be baited for different species, according to the baiting lure, and used several times. The scientific literature provides a very large number of papers about the trapping performance of different trap types, suggesting that successful pest detection and monitoring needs a species-specific trap design (Nageleisen & Bouget, 2009). The factors affecting pheromone trapping systems and trapping efficiency are discussed with regard to different application objectives.

Pathways and commodities

Although the range expansion of native species is of interest, here we consider only the establishment of truly exotic species, not simply interceptions, as suggested by Kirkendall & Faccoli (2010) and Marini *et al.* (2011). While the majority of introductions of alien insects to Europe are via trade in ornamental plants (Kenis *et al.,* 2007; Roques *et al.,* 2009), bark and ambrosia beetles travel mainly in fresh – often undebarked – wood and timber. Ambrosia beetles may also travel in wooden packaging materials (such as crates, dunnage and pallets) made with recently infested fresh wood (Haack, 2001; Allen & Humble, 2002; Colunga-Garcia *et al.,* 2009; Haack & Petrice, 2009). Only a few scolytid species are likely to be transported in plants or plant parts. The cut stems of *Dracaena,* which are shipped to Europe from Central America, are frequently infested with tropical *Xyleborus* species; seeds and nuts with *Coccotrypes, Dactylotrypes* and *Hypothenemus*; and the orchids with *Xylosandrus morigerus* (Kirkendall & Faccoli, 2010). *Hypocryphalus scabricollis* probably entered Malta with exotic *Ficus* trees from southern Asia (Mifsud & Knížek, 2009).

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Flatheaded borers or jewel beetles (Coleoptera: Buprestidae)

(A. Roques)

Introduction

Although only two species of exotic buprestids of minor importance (*Buprestis decora* and *Chrysobothris dorsata*) have so far established in Europe (Denux & Zagatti, 2010), other species in this family have to be considered as potential threats to European forests, taking into account the extensive damage caused by the emerald ash borer, *Agrilus planipennis*, in North America since its accidental introduction in the early 2000s (Cappaert *et al.*, 2005). This species then invaded the Moscow area in Russia, and is currently expanding westwards towards Central Europe (Baranchikov *et al.*, 2008). Besides *A. planipennis* (A2 List), a congeneric species, the bronze birch borer, *A. anxius*, has recently been recommended for regulation (A1 List). Moreover, eight other *Agrilus* buprestids are considered to be aliens in North America (Jendek & Grebennikov, 2009), indicating the considerable invasive potential of this genus. Species in the genera *Buprestis* and *Chrysobothris* may also be of concern. Most adult buprestids typically use host volatiles to locate stressed trees (Crook *et al*., 2008; De Groot *et al*., 2008), and a number of them have been shown to use visual cues for mating as well as for host location (Lelito *et al.*, 2008). Thus coloured visual traps, unbaited or baited with plant volatiles, have been developed extensively to detect and monitor these species (Braman *et al.*, 2003; Sakalian & Langourov, 2004; Miller, 2006; Crook *et al.*, 2008; Francese *et al.*, 2008; Corte *et al.*, 2009).

Detection and monitoring

Infested material (e.g. logs, plants for planting, bonsais) is difficult to detect by visual inspection on arrival. After arrival, detection methods rely on adult trapping rather than surveys of larval damage, as no visible symptoms can be observed in the first year of infestation. Contact sex pheromones and aggregation pheromones have been identified in adults of *A. planipennis* (Bartelt *et al.*, 2007; Lelito *et al.*, 2009; Silk *et al.*, 2009), but their practical use for early detection is limited. To locate early stage infestations of *A. planipennis* at sites with low levels of infestation, the use of purple prism-shaped sticky traps is recommended (Crook *et al.*, 2008; Francese *et al.*, 2008). Traps are baited with a blended lure consisting of 80% manuka oil, a steam distillate from the New Zealand manuka tea tree (*Leptospermum scoparium*), and 20% phoebe oil, a steam distillate from Brazilian walnut (*Phoebe porosa*), which both contain volatiles found in green ash. A release rate of 50 mg per day is recommended (Crook *et al.*, 2008; Marshall *et al.*, 2010). Traps should be sited preferably on ash trees located along woodland edges, in open areas, or in open stands such as in parks, but also in other sites at high risk due to the transport of firewood, such as camping grounds and recreation areas. Traps should be hung in the canopy of dominant ash trees, as high as possible (and at least 6 m from the ground), on the southern side of the tree (Francese *et al.*, 2006). Large traps appear to be more useful as detection tools than smaller ones (Francese *et al.*, 2010b).

Dark and light green prism-traps with a peak reflectance of 540 nm can also be used; in a recent study these caught two to three times as many emerald ash borers as purple traps either in mid-canopy (13 m) or lower canopy (6 m), although there was no difference between traps hung at 1.5 m above ground (Francese *et al.*, 2010b). However, the sex ratio on green traps, unlike purple traps, is heavily skewed toward males (Crook *et al.*, 2008; Francese *et al.*, 2010a,b). The addition of (*Z*)-3-hexenol (*Z*3-6:OH) also induces a strong male-biased response, whereas manuka oil attracts both sexes equally (Grant *et al.*, 2010).

Other detection methods, which are apparently less efficient at low emerald ash borer population densities, include girdled (stressed) detection trees which are peeled and examined for larvae (McCullough & Siegert, 2006); sticky bands placed on trunks or logs to trap landing adults (Lyons *et al.*, 2009); and sticky-leaf traps made of live ash leaves covered with spray-on adhesive to which a dead emerald ash borer male has been pinned (Lelito *et al.*, 2008). The solitary parasitoid wasp *Cerceris fumipennis*, a buprestid-hunting Crabronidae (Hymenoptera), also has the potential to detect low levels of the emerald ash borer in the early stages of an infestation, and is used for that purpose in some parts of the North-Eastern USA (Marshall *et al.*, 2005).

The use of purple traps also allows a related *Agrilus* species to be detected: the gold-spotted oak borer (*A. coxalis*), a recent invasive buprestid in California (Coleman & Seybold, 2008). However, other species of *Agrilus* have been detected using yellow traps in Italian hazelnut stands (Corte *et al.*, 2009) and in Bulgaria (Sakalian & Langourov, 2004). *Agrilus cyanescens* showed a strong response to blue-coloured sticky traps on which a dead male emerald ash borer was pinned (Lelito *et al.*, 2008). No detection methods other than visual inspection exist so far for *Agrilus anxius*.

Purple traps have also been proven to constitute an efficient tool to detect species in the genus *Chrysobothris*, such as the flatheaded appletree borer *C. femorata* (Oliver *et al.*, 2004; Hansen *et al.*, 2009). Other trap colours may be effective for some species. Pyramid-shaped traps painted in grey detected *Buprestis lineata* and *Chrysobothris* spp. in Georgia, USA (Braman *et al.*, 2003). The steelblue jewel beetle, *Phaenops cyanea*, was trapped by black foil bands in Poland (Sowinska & Janiszewski, 2007), whilst rectangular silver aluminium foil traps caught the poplar stem borer beetle, *Melanophila picta*, in Iran (Akbarian *et al.*, 2006).

Besides visual trapping, multiple-funnel traps baited with tree volatile compounds or sex pheromones of associated

xylophages may be used to detect buprestid species (Costello *et al.*, 2008). Traps baited with (−)-alpha-pinene were attractive to *Buprestis lineata* in the USA (Miller, 2006), whereas Pheroprax pheromone traps set for the bark beetle *Ips typographus* in Slovakia additionally caught *Anthaxia quadripunctata*, *A. helvetica*, *A. cyanea*, *Phaenops cyanea*, *Melanophila knoteki*, *Buprestis haemorrhoidalis* and *Chrysobothris affinis* (Zach, 1997).

Once adults have been trapped, or an infested tree detected, the delimitation of the invaded area is based on a visual estimation of damage. For *Agrilus planipennis*, a visual survey has to be carried out until infested trees are no longer found, and at least for a distance of 3 km beyond the initial trap catch or infested tree detection. Visual survey may detect only trees that have been infested for 3 years or more. The following visual symptoms should be looked for: canopy dieback, epicormic branching, woodpecker feeding sites, presence of D-shaped exit holes of 3–4 mm in diameter and bark cracks with serpentine larval galleries. On large trees, symptoms may be present only in the upper canopy in the early stages of infestation, and tree climbers may be needed to provide a more intense inspection than is possible by visual survey from the ground.

In the USA, the following survey guidelines were recommended for the emerald ash borer in 2010 (USDA APHIS PPQ, 2010). A grid-based survey should be carried out within an 80 km band surrounding the periphery of the generally infested area. Activities include the development of a trapping grid (purple prism traps baited with a blend of manuka oil/phoebe oil – see above) and identification of high-risk sites, the selection of trees and placement and maintenance of traps, a visual survey of the environment in proximity of the traps for damage symptoms, and visits to high risk locations. A trap should be placed within each 2.5 × 2.5 km grid square where ash trees are present. Traps should be spaced as uniformly as possible within the grid, taking into consideration accessibility and the presence and condition of ash trees. High-risk sites in the inner boundary of the band survey should be targeted and prioritized as trap locations. Examples of high-risk sites are locations where declining ash trees are observed with at least two of the symptoms indicated in the above section on the delimitation of the infested area: camping grounds and recreation areas, nurseries, sawmills, arborist/landscape firms and firewood dealers, and recently landscaped residential and commercial properties. The target density for a selected site is at least one trap per 2.5 km^2 and up to four traps per site. However, sites associated with known pathways from the generally infested area may be surveyed using up to 4 traps per 2.5 km^2 and up to 16 traps per site.

Pathways and commodities

Transportation with wood for industry and firewood, especially for the emerald ash borer, is the most likely pathway. Larvae can also be introduced with bonsais. Adults are active flyers. The main hosts are ash (*Fraxinus* spp.) for emerald ash borer (*Agrilus planipennis*) and birch (*Betula* spp.) for the bronze birch borer (*Agrilus anxius*).

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Leaf beetles (Coleoptera: Chrysomelidae)

(A. Battisti)

Introduction

Leaf beetles (Coleoptera: Chrysomelidae) are important pests of cultivated plants in agriculture, and especially in Europe they have recently become very important, one example being the spread of *Diabrotica virgifera* subsp. *virgifera*. In addition, they have been often used for classical biological control of weeds, including trees such as *Tamarix* in Western USA, with the subsequent risk of becoming established as invasive on other native plants. A number of detection and monitoring techniques have been developed, mainly in North America and Europe, to survey populations. In this family, visual and olfactory cues are used for host-finding (Stenberg & Ericson, 2007). The beetles mainly have a diurnal activity (Boiteau *et al.*, 1979).

In North America, the bean pod mottle virus is associated with the bean leaf beetle *Cerotoma trifurcata* (Forster). The virus may enter soybean plants through insect feeding. The principal vector for this virus is the bean leaf beetle. When beetles feed on soybean leaves, they produce a small amount of regurgitated plant material that may contain virus particles (Rice *et al.*, 2010). Management of the beetles may reduce the risk of transmission (Krell *et al.*, 2004). Potato Andean latent tymovirus is transmitted by the potato flea beetles *Epitrix* spp. at high population densities (EPPO/CABI, 1997).

Detection and monitoring

The aggregation pheromone (male-specific) of the cereal leaf beetle (*Oulema melanopus*), an invasive alien species in North America, has potential as a monitoring tool for early detection of the beetles as they move from their overwintering sites into newly planted cereal crops in spring (Rao *et al.*, 2003). Similar pheromones are emitted by male *Galerucella calmariensis* and *Galerucella pusilla*, introduced for weed control in North America, while feeding on host foliage (Bartelt *et al.*, 2006). The combination of the pheromone and the green leaf odour blend could be a useful attractant in detecting the presence of the *Tamarix* biocontrol agent *Diorhabda elongata* in stands of saltcedar newly colonized by the beetle (Cosse *et al.*, 2006). Cucurbitacins have also been identified as kairomones for *Diabrotica* beetles (Metcalf *et al.*, 1980).

Trap designs baited with synthetic sex pheromone have been optimized for trapping of the western corn rootworm *Diabrotica v. virgifera* LeConte, which has recently been introduced into Europe. The best trap design has proved to

be the sticky 'cloak' trap, which catches only males, and is being used in many countries of Europe for detection and monitoring the spread of this new pest. The range of attraction of the pheromone traps was estimated to be <10 m. The performance of yellow sticky plates was insignificant compared with the activity of the pheromone baited traps and the yellow colour had no discernible effect on catches in pheromone traps. The floral lure containing 4-methoxycinnamaldehyde and indole also proved to be attractive to both females and males in Europe. Since the yellow colour slightly increased catches by the floral lure, a yellow sticky 'cloak' trap has been developed. Pheromone baited traps caught approximately four times more beetles than the floral baited traps, but the latter appeared to be preferentially attractive to females. When placed in the same trap, the pheromone and floral lures did not interfere with each other's activity (Tóth *et al*., 2003).

Andow *et al.* (1990) have used a number of methods to delimit the area infested by the cereal leaf beetle in the USA. Pheromones and other cues are used in monitoring programmes, although the response of the beetles is not constant through the season and may depend on the host plant on which they feed. Bartelt *et al.* (2008) present a model for *Galerucella* pheromone-mediated host colonization after dispersal depending on pheromone biology, diapause, photoperiod and host quality.

A number of classical monitoring methods are available for leaf beetles, such as vacuum sampling, sweeping, and ground cloth methods (Ruesink & Haynes, 1973; Turnipseed, 1974). In addition, emergence traps from soil have also been used (Boiteau *et al.*, 1979; Jeffords *et al.*, 1983). Monitoring methods for the cereal leaf beetle (Bai *et al.*, 2001) have been studied intensively. Oviposition traps made of cylinders with different clay aggregates have been used to recover eggs of *Diabrotica virgifera* in North America (Mulock *et al.*, 1995).

Sticky traps of various types associated with pheromones appear to be the best methods to detect leaf beetles. If no lures are available, traditional sampling (e.g. emergence traps, vacuum, sweeping) is a reliable alternative, as well as trap crops for the Colorado beetle *Leptinotarsa decemlineata*.

Pathways and commodities

Leaf beetles are generally associated with pathways in both Japan (Kiritani & Yamamura, 2003) and Europe (Roques *et al.*, 2008), although specific data is largely missing. *Diabrotica speciosa* (EPPO, 2005) has been associated with the soil pathway, while *Gastrophysa polygoni*, which feeds mainly on knotweeds and wild buckwheat, was probably introduced with weed-contaminated seed (Lesage & Majka, 2009). The palm leaf beetle *Brontispa longissima*, originating from the Pacific islands, is a newly invasive pest in China and is associated with the plants for planting pathway (Lu *et al.*, 2004).

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Longhorn beetles (Coleoptera: Cerambycidae)

(E. Petrucco Toffolo)

Introduction

Most species of Cerambycidae attack dying or dead trees and have an ecologically important function, but some are able to invade healthy or weakened trees, eventually killing them due to girdling of the phloem as a result of extensive larval feeding under the bark. The attack by cerambycids may cause secondary economic losses on naturally damaged or felled timber (Evans *et al.*, 2004). Some species are notable causes of tree death in the urban and peri-urban environment (Allison *et al.*, 2004), such as *Anoplophora* spp., which pose an enormous threat to urban and suburban forests in areas where they are introduced (Haugen, 2000; Nowak *et al.*, 2001). Based on this information, *A. glabripennis* is now listed as a quarantine pest in North America and in the European Community (USDA-APHIS [USDA Animal and Plant Health Inspection Service], 1998; EU Council Directive 2000/29). The Cerambycidae can be vectors of very harmful pests, such as the pine wood nematode *Bursaphelenchus xylophilus* transmitted by *Monochamus* spp. (Schröder *et al.*, 2009).

Detection and monitoring

Notwithstanding the large number of studies that have been undertaken to try to find an efficient method to survey cerambycids, the results are not yet satisfactory. Research on the chemical ecology of over 70 species has revealed many examples of attractive kairomones (such as floral volatiles, smoke volatiles, trunk and leaf volatiles and bark beetle pheromones), repellents and deterrents, oviposition stimulants, short- and long-range sex pheromones and tree defensive substances (Allison *et al.*, 2004). Volatile sex or aggregation pheromones produced by males have been identified in five species (Ray *et al.*, 2006). For *Anoplophora* and *Monochamus*, two genera associated with damage to broadleaf and coniferous trees, respectively, there have been recent advances in the development of an efficient lure that can be efficiently used for monitoring.

Specifically, there has been great interest in identifying pheromones of *Anoplophora* spp. to facilitate their early detection (Haack *et al.*, 2010). Preliminary experiments have shown that male orientation is influenced by volatiles released by females (Li *et al.*, 1999). Further investigations revealed potential pheromones produced by male *A. glabripennis* (Zhang *et al.*, 2002). Greenhouse experiments have shown that adults of *A. glabripennis* are, to some extent, attracted to traps baited with a combination of the male-produced pheromone blend and (*Z*)-3-hexen-1-ol (Nehme *et al.*, 2009). Males attempted to mate when in contact with a surface coated with a synthetic mixture of female cuticular extracts, indicating that the blend effectively elicits copulatory behaviour in males (Zhang *et al.*, 2003). Recently, a contact sex pheromone was also discovered in *A. chinensis* females (Mori, 2007; Yasui *et al.*, 2007). However, no long-range pheromone has yet been found, although male-produced short-range pheromones and female-produced contact-recognition pheromones have been identified. In other species, the only option is to use generic kairomones, but some species show a limited response and remain difficult to detect (Allen & Humble, 2002). Several studies have investigated the role of plant volatiles – especially those extracted from *Acer negundo* – in host finding and acceptance, with the goal of finding compounds that could be used as lures in trapping programs (Li *et al.*, 1999; Wen *et al.*, 1999; Jin *et al.*, 2004; Zhang *et al.*, 2008; Hu *et al.*, 2009; Smith *et al.*, 2009).

Whereas generic lures do not work for *Anoplophora* adults, a mix of host volatiles (such as α-pinene, ethanol or 3-carene) is a powerful attractant for Asian and North American species of *Monochamus* (McIntosh *et al.*, 2001; Morewood *et al.*, 2002; Miller, 2006; Fan *et al.*, 2007; Costello *et al.*, 2008). The addition of bark beetle pheromones such as ipsenol increased the trap efficiency (Allison *et al.*, 2001), with some exceptions (Fan *et al.*, 2010), possibly depending on population density (Miller, 2006). In the pine sawyer beetle *Monochamus galloprovincialis*, the male-produced aggregation pheromone (2 undecyloxy-1-ethanol) has allowed high numbers of captures of both sexes when combined with other compounds (Pajares *et al.*, 2010). Numerous experiments have addressed the performance of different models of trap, although there is no clear conclusion. Cross-vane and multi-funnel traps are mostly used to survey cerambycid species; visual cues associated with trap colour appear to be important, as black traps are more

efficient. Multi-funnel traps baited with a specific commercial kit (Galloprotect 2D®; Pajares *et al.*, 2010) was the most effective combination to catch adults of *Monochamus galloprovincialis* (Rassati *et al.*, 2012).

For those species lacking efficient trapping systems, the most common detection technique is visual inspection of plant material. *Anoplophora* species have been well studied in this respect, especially for the detection of oviposition pits and emergence holes. In *A. glabripennis*, detection is particularly challenging because most infestations begin near the top of the tree and are hidden by foliage. Different methods of inspection have been implemented, and vary in their efficiency and expense. These include ground surveys where the trunk is visually examined and upper parts of the tree are checked by inspectors using binoculars, or using elevators and tree climbers to survey the upper canopy. In addition, methods have been developed to detect larvae in wood using non-contact ultrasounds (Fleming *et al.*, 2005) as well as by using the acoustic signatures of feeding larvae (Mankin *et al.*, 2008).

Pathways and commodity

The cryptic wood-boring habits of many cerambycids make them ideally suited for introduction as exotics in wood products, dunnage and nursery stock. Many cerambycid species are transported between countries – mainly from Asia to the USA and Europe – inside live plants and wood packing material used in international cargo (Hu *et al.*, 2009; Haack *et al.*, 2010). *Anoplophora* spp. are most likely to move as eggs, larvae or pupae in woody planting material, including bonsai plants, and possibly in packing material. In particular, the bonsai trade is known to be responsible for the introduction of the citrus longhorned beetle (*Anoplophora chinensis*) to France, Italy and USA in recent years (Hérard *et al.*, 2005). Another important pathway for cerambycids is wood packaging material, usually produced from low-grade wood of various tree species, often with bark and portions of vascular cambium (Clarke *et al.*, 2001).

More than 200 distinct interceptions of *A. glabripennis*, *A. chinensis* or *Anoplophora* spp. were made in 18 countries from 1980 to 2008 (Haack *et al.*, 2010). *A. glabripennis* was intercepted in wood packing material associated with imports such as steel, ironware, tiles and quarry products, as well as in live woody plants such as bonsai and nursery stock, whereas most *A. chinensis* interceptions were on live plants (bonsai and plants for planting; Haack *et al.*, 2010). Most interceptions of *A. glabripennis* originated from China, whereas live plants infested by *A. chinensis* originated from China, Japan and South Korea (Haack *et al.*, 2010). Although port inspectors often target high-risk cargo, overall inspection rates are low worldwide (Haack *et al.*, 2010). Moreover, it is difficult to discern actual trends in the interception data, given the annual variation in trade volume, trading partners, international regulations and country inspection rates. However, it is clear that both species continued to move in international trade despite the implementation of measures to reduce their occurrence (Haack *et al.*, 2010).

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Weevils (Coleoptera: Curculionidae)

(E. Petrucco Toffolo)

Introduction

Weevils are a heterogeneous group, and feed mainly on leaves, fruits, seeds and roots (Hill, 1997). They are important pests of herbaceous crops, but there are numerous species that are harmful to woody plants in orchards, plantations and forests. The damage can be caused by larvae as well as adults. Many weevils are cryptic and nocturnally active, and collecting them can be difficult. As a result, exotic pest weevils entering an area may remain undetected for many years until their population builds up to economically important levels (Bloem *et al.*, 2002). For example, the eucalyptus weevils *Gonipterus gibberus* and *Gonipterus scutellatus* are not important pests in their native country (Australia), while they have become important defoliators of eucalyptus in other parts of the world (EPPO, 2005). The palm weevil *Rhynchophorus palmarum* may transmit the nematode *Rhadinaphelenchus cocophilus*, which causes red-ring disease of oil palm in tropical America (Howard *et al.*, 2001).

Detection and monitoring

Weevils generally respond to a blend of volatiles, including host odours and pheromones active at both short and long distance. As there is a large variation among species, it is difficult to make the results apply to all species. Males of the pepper weevil *Anthonomus eugenii* produce an aggregation pheromone that attracts both sexes (Capinera, 2005). The pheromone was identified by Eller *et al.* (1994) and further experiments were carried out to increase its efficiency in field trials (Bottenberg & Lingren, 1998) until a pheromone trap became available (Webb *et al.*, 2010). Numerous studies were also carried out for *Anthonomus grandis*, the boll weevil. Traps baited with the synthetic pheromone of the boll weevil are used extensively to detect and monitor populations (Hardee & Mitchell, 1997). Judicious placement of traps in locations protected from prevailing winds should improve detection efficiency in areas where the early warning of weevil presence is critical (Sappington & Spurgeon, 2000). Male American palm weevils, *Rhynchophorus palmarum*, emit a volatile aggregation pheromone, rhynchophorol (Rochat *et al.*, 1991), but attraction to traps baited with pure rhynchophorol is low; the addition of plant material increases catches considerably (Oehlschlager *et al.*, 1993). Saïd *et al.* (2005) found that a mixture containing 5–10% acetoin in ethyl acetate acted in synergy with the aggregation pheromone of *R. palmarum*; food-baited pheromone traps also showed higher performance than the pheromone trap for other species of palm weevil, such as *Rhynchophorus ferrugineus* (Faleiro, 2006). A similar situation was observed for *Conotrachelus*

nenuphar, the plum curculio. Traps baited with benzaldehyde (synthetic fruit volatile) plus a pheromone (grandisoic acid) placed at borders of *C. nenuphar* overwintering sites can be a valuable tool for monitoring the beginning, peak and end of adult immigration into apple orchards (Piñero *et al.*, 2001; Leskey & Wright, 2004); an alternative is the use of odour-baited trap trees as sentinels to monitor the *C. nenuphar* (Prokopy *et al.*, 2004).

For other species, such as the Andean potato weevils *Premnotrypes* spp. and *Hypera* weevils, the compounds implicated in host finding still remain unknown (Kühne, 2007). In field and laboratory experiments using potato leaves as bait, adults of *P. vorax* and *P. suturicallus* were significantly attracted to these baits (Valencia, 1989). However, it has been speculated that only foliage together with adults feeding on them may attract other adult weevils (Heath *et al.*, 2001; Kühne, 2007). A generic trapping study on *Hypera* weevils, carried out in several wild and cultivated habitats in North Florida, obtained interesting results. Four new important agricultural species were recorded for the first time in the state: *Hypera meles*, *H. nigrirostris*, *H. punctata* and *Sitona lineatus*. Three types of unbaited trap were tested: black pyramidal trap; yellow pyramidal trap; and passive circle trap (Bloem *et al.*, 2002). The types of trap differ according to the species monitored. The pyramidal trap and the panel trap are commonly used in monitoring *C. nenuphar* (Piñero *et al.*, 2001), although Leskey & Wright (2004) found screen traps to perform better. Plastic bucket traps are used for palm weevils (Rochat *et al.*, 2000; Abraham *et al.*, 2001); sticky or multifunnel traps for forest Curculionidae such as *Pissodes* spp. (Chénier & Philogène, 1989); and sticky traps for monitoring *Anthonomus eugenii* (Capinera, 2005).

A monitoring system based on acoustic sensors can be used for early detection of *Rhynchophorus ferrugineus* larvae in the interior of palms (Potamitis *et al.*, 2009; Hussein *et al.*, 2010).

Statistical methods such as generalized linear models have been tested to identify the optimal sample size for accurately estimating populations of the Eucalyptus leaf beetle (Candy, 2000).

Pathways and commodities

The pathway is related to the life-cycle of the species: many weevil beetles species are transported between countries inside fruits or seed containing the larvae or pupae. For eucalyptus and pine weevils, the risk is related to adults, larvae or eggs present on plants for planting. For example, the red palm weevil *R. ferrugineus*, a native of South Asia, over the past two decades has invaded several Middle Eastern countries, from where it has moved to Africa and Europe, mainly due to the movement of infested planting material (Faleiro, 2006). Another important pathway for some species is in the accompanying soil, which may contain larvae or pupae.

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Cone and seed flies (Diptera: Anthomyiidae)

(A. Roques)

Introduction

Cone flies, also called cone maggots, belong to the genus *Strobilomyia* (Diptera: Anthomyiidae). They are among the most serious cone-and-seed pests of conifers (Turgeon *et al.*, 1994). Their larval instars develop exclusively in cones of Pinaceae, where they feed on cone tissues and seeds (Michelsen, 1988). Until the 1980s, precise knowledge of the specific distribution and life cycle of *Strobilomyia* species was limited by taxonomic uncertainties, but 20 *Strobilomyia* species have now been recognized (Michelsen, 1988; Roques *et al.*, 1995; Roques *et al.*, 2003; Sachet *et al.*, 2006), of which 12 have been recorded on larch (*Larix*), 5 on fir (*Abies*), and 3 on spruce (*Picea*). The geographical distribution of the genus is large, including boreal forests and alpine regions of the Palearctic and the Neartic, but only three species are native to Europe. A trans-Beryngian species, *S. viaria* (Huckett; = *Lasiomma melaniola* Fan = *Strobilomyia melaniola* Fan), is presently included in the EPPO A2 list of pests recommended for regulation. Mature flies locate the host cones during the day, using a combination of visual and olfactory cues (Roques, 1986a). This behaviour has enabled the development of a number of detection and monitoring techniques in Europe, China and North America (Roques, 1986b; Yao *et al.*, 1991; Chau, 1993; Roques *et al.*, 1996; Yan *et al.*, 1997, 1999, 2002).

Detection and monitoring

Infested cones are difficult to identify based on external observations, and cones need to be cut into slices. Larval damage is indicated by large galleries filled with resin but not frass, which spiral around the cone axis, sometimes entering it (Skuhravá & Roques, 2000).

No sex pheromones have yet been identified in cone flies. Although not specific, coloured sticky traps are highly efficient for detecting adult flies. A horizontal fluorescent yellow plate, acting as a nutritional (flower)-type stimulus, combined with a vertical plate coloured in fluorescent yellow with purple vertical stripes, mimicking the contrast between cones and foliage, efficiently traps males of the European larch cone flies (Roques, 1986b; Da Ros, 1997; Olenici *et al.*, 2001). Sexually immature females of six Asian species of larch and spruce cone flies are trapped in signficant numbers by deep blue cups hanging upside down about 2 m above ground level (Roques *et al.*, 1995; Yan *et al.*, 1997, 2002). Blue cups are similarly efficient in trapping immature females of a North American larch cone fly, *S. laricis* (Chau, 1993) as well as European larch cone flies (Roques, unpublished data). Baiting visual traps with monoterpene blends extracted from healthy cones may increase specificity in captures, but the results are not yet conclusive (Yan *et al.*, 1999). Because of the lack of specificity of visual traps, genitalia dissection must be used systematically for the accurate identification of trapped flies (Michelsen, 1988; Roques *et al.*, 2003).

Visual traps have been used in monitoring programmes in Europe (Jenkins & Roques, 1993; Olenici *et al.*, 2001), North-Eastern China (Roques *et al.*, 1995; Yan *et al.*, 1997) and Canada (Chau, 1993). Models have been developed for larch stands in Europe to estimate the level of cone damage to be expected for different cone crop sizes with regard to the number of flies trapped by a set of horizontal and vertical traps (Roques, unpublished data). A more classical method consists of taking a random sampling of cones during the spring development period to measure the number of cones infested with fly eggs and/or larvae (Ruth *et al.*, 1982; Roques, 1988; Sweeney *et al.*, 1990; Yao *et al.*, 1991; Turgeon & de Groot, 1992; Liu & He, 1994; McClure *et al.*, 1996). The number of cones sampled has to be adjusted in relation to the size of the cone crop.

Pathways and commodities

Immature cones are unlikely to be imported either alone or with conifer seedlings. Thus eggs and larvae are unlikely to be disseminated because they occur only in cones. Bonsais may provide an exception to this because they may bear developing cones. Pupae can be disseminated with the soil of potted conifer seedlings. Larch cone flies native to the Alps were probably disseminated in lowland larch plantations of Western Europe and the UK with this plants for planting pathway (Roques, unpublished observations).

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Fruit flies (Diptera: Tephritidae)

(S. Quilici and P. Donner)

Introduction

Fruit flies (Diptera Tephritidae) are worldwide pests of fruit, and many species in this family are listed as quarantine pests for Europe. Major fruit-producing countries in the world (the USA, Australia, New Zealand) have been developing surveillance networks for this type of pest over a long period. Such networks, which are quite limited on European territory at the moment, would be very useful to allow for the early detection of exotic fruit fly species.

On a worldwide basis, the fruit fly species representing the highest risk are listed below.

(a) *Bactrocera* spp. from Asia (e.g. *B. dorsalis*, *B. zonata*, *B. correcta*, *B. cucurbitae*) and now also *B. invadens* (which has spread through most of sub-Saharan Africa in recent years), are a major threat for the USA as well as for Europe. In Europe, *B. zonata* is also a target species.

(b) *Ceratitis capitata* (Medfly) is still a quarantine pest for the USA and New Zealand, while a few other African *Ceratitis* spp. are important for the USA and Europe.

(c) To a lesser extent, a number of *Anastrepha* spp. are a threat, mostly for the USA.

There are no records of Tephritids vectoring plant diseases, although human pathogens can be carried by Medfly (Sela *et al.*, 2005).

Detection and monitoring

Depending on the country considered, the organization of fruit fly surveillance follows a national or regional scheme. The New Zealand trapping network is national, while in the USA surveillance programmes have developed independently in each state. In Australia, the network includes traps funded by the Australian government (Department of Agriculture, Fisheries and Forestry, DAFF) and maintained by the different state governments (OCPPO, 2010). State governments generally coordinate their surveillance efforts at the

national level (for instance through the Office of the Chief Plant Protection Officer, OCPPO, in Australia). In the USA, national and cooperative state/federal trapping protocols have been developed to guide surveillance activities, and federal initiatives helped develop a surveillance network in Puerto Rico. The aim is to detect introductions of *B. cucurbitae*, *B. dorsalis* and *C. capitata* and also to survey for endemic species, *A. suspensa* and *A. obliqua*, to prevent their entry into the continental USA (Burnett *et al.*, 2006). In Spain, regional networks have been set up in Catalonia, Valencia province and Andalusia (Combo Suarez, 2010).

Trapping systems

The *Trapping Guidelines for Area-wide Fruit Fly Programmes* (IAEA, 2003) and the Annex 1 to the International Standard for Phytosanitary Measures (ISPM) no. 26 (IPPC/FAO, 2008) provide general information and recommendations on fruit fly trapping. These documents give detailed recommendations on the most important features of a detection trapping programme, regarding trap types, lures and trap densities as well as trapping procedures. The United State Department of Agriculture (USDA) has also published a *National Exotic Fruit Fly Trapping Manual* (Anonymous, 1991), which provides detailed recommendations for surveillance of the most important fruit flies for the USA. Florida and California have also developed trapping manuals providing similar recommendations (Gilbert *et al.*, 2005; Anonymous, undated). In Australia, a fruit fly code of practice (COP) is under revision and should be published in the near future by the Office of the Chief Plant Protection Officer (OCPPO, 2010).

Recently, new perspectives have arisen in Australia (Liu *et al.*, 2009) which could lead to a substantial improvement in trapping systems through the use of fruit fly traps incorporating a sensor that can capture high-quality images and techniques for automatically detecting the presence of fruit flies.

Lures

For all species whose males respond to para-pheromones, these compounds are widely used as lures in surveillance programmes. This is the case with trimedlure (or alternatively Capilure) for certain *Ceratitis* spp., methyl-eugenol for some *Bactrocera* spp. and cue-lure for some other *Bactrocera* spp. For species whose males do not respond to para-pheromones, general food attractants, active for both sexes, such as Torula Yeast, or synthetic lures such as '3 lures', 'two-components lure' or ammonium salts are used. In a few cases true pheromones are used, such as spiroketal for *Bactrocera oleae*. The availability of lures for species included in the lists of Council Directive 2000/29 and those of EPPO is given in the supporting information available on http://archives.eppo.int/files/pratique_42_1/augustin.xls.

Traps

Various trap types have been developed in the past and a few are still widely used worldwide in different surveillance programmes (IAEA, 2003). Para-pheromones are generally used with dry traps, sticky traps or sticky panels. Dry traps include Steiner-type models, such as the Lynfield trap used in New Zealand (with two holes on the side), or Nadel-type traps (with 3 or 4 lateral holes), which also have many local versions worldwide. Delta traps with a sticky base are also employed in various programmes, such as the Jackson trap, widely used in the USA. For liquid attractants, traps are generally of the McPhail type, of which various plastic versions are available, for instance, the 'Multilure' trap MLT (Better World Manufacturing Inc., Fresno, CA, USA). Other traps, which are hybrids between the McPhail and Nadel types, allow the use of dry or liquid attractants (for instance, the 'Tephri-trap', Sorygar, Spain). Sticky panels, such as the 'Champ trap' (Seabright, Albany, CA, USA) are also regularly used in the USA.

Areas surveyed and trap density

In all countries where fruit fly surveillance is operating, trapping networks are risk-based. Traps are set up in areas considered to be at high risk: ports of entry, populous regions and commercial production areas in climatically suitable areas. As recommended by the IAEA (2003), urban areas and points of entry are considered to pose a higher risk than rural residential areas and host orchards. In Australia, a National Exotic Fruit Fly Surveillance Programme (NEFFS) was specifically implemented in the vicinity of all ports of entry to the country in 1996 in addition to the surveillance of horticultural orchards and main cities (OCPPO, 2010). In New Zealand, a constant trap density is deployed at around 3000 trapping sites concentrated in points of entry or areas with the highest host fruit production, with 2952 sites situated in the North Island (2107 in Auckland) and 506 in the South Island (Stephenson *et al.*, 2003). Similarly, in the USA, the trapping effort is concentrated at points of entry and urban areas. Traps are, for instance, deployed in the vicinity of maritime ports in Georgia, Mississippi, Alabama, Louisiana and South Carolina, and at Mexican transit corridors in Arizona and New Mexico. In Texas, some traps are set up at land border ports as well as in the vicinity of international seaports. In California as well as in Florida, double the density of traps are placed at points of entry and urban areas compared with rural residential areas (Burnett *et al.*, 2006; Hoffman, 2010). For example, Florida uses different trap densities based on three risk levels: (a) international ports of entry, (b) areas presenting high risk of illegal fruit introduction, and (c) private houses, businesses or locations situated close to host production areas (Clifton & Cusano, 2009). In California, trap density varies between 0.8 and 1.9 traps per km^2 in urban areas, and between 0.4 and 1.5 traps per km^2 in residential areas, but, depending on the risk level, the trapping system and the target species, the density may vary from 0.06 to 8.5 traps per km^2 (Hoffman, 2010). Generally, for a given state, a distinction is also made between counties with different levels of risk, associated for instance with climate suitability as well as the area

of fruit crops. In Florida, California and other states, some counties trap only on a seasonal basis.

In the USA, as well as in Australia and New Zealand, a positive trap detection triggers enhanced surveillance to determine the area of infestation with supplementary trapping and larval searches. Additional catches trigger an eradication procedure. The number of flies caught or the presence/absence of larvae are important parameters to consider, and may vary according to each specific programme and target species (for instance, the finding of 2 flies within 5.6 km, or larvae originated from them, for the Oriental fruit fly in Florida). The size of the delimitation area may also vary with the programme: in the USA it is 210 km^2.

Costs

In New Zealand, the surveillance programme is operated and funded by the government at a cost of 760 000 EUR a year (http://www.biosecurity.govt.nz). In the USA, the cost of the exotic fruit fly surveillance programme was evaluated at 15.8 million EUR (Burnett *et al.*, 2006), but it is not clear if eradication costs were included in this assessment. In Australia, the total budget for exotic fruit fly trapping, funded by the OCPPO as part of the NEFFS, reached 2.2 million EUR for the period from July 2003 to June 2008, i.e. 473 000 EUR a year. In addition to this, the long-term containment strategy for exotic fruit flies in the Torres Straits Islands cost 700 000 EUR for the same period, i.e. 137 000 EUR a year. The total budget for all Australian fruit fly activities for the same period was 104 million EUR, i.e. 20.3 million EUR a year (OCPPO, 2007).

Application to specific groups

Ceratitis. Ceratitis species are surveyed using the para-pheromone trimedlure in all countries. However, a powerful food attractant, the female biased Biolure ('3C'), may enable the detection of *C. capitata* 4–6 weeks earlier than trimedlure (IAEA, 1999; Miranda *et al.*, 2001; Burnett *et al.*, 2006). This attractant may be used with trimedlure for detection or delimitation, e.g. in California and Florida. The recommended ratio is 3–4 female-biased traps (Multi Lure Trap/3C) for 1 male trap (Jackson trap/Trimedlure; IAEA, 2003; Burnett *et al.*, 2006).

Bactrocera. A large number of *Bactrocera* spp. are detected by trapping males using methyl-eugenol or cue-lure, depending on the species. However, the males of some *Bactrocera* spp. do not respond to any of these lures. *Bactrocera oleae* constitutes a particular case: in this species, the males responding to the natural pheromone (spiroketal) emitted by the females. The density of traps used depends on the attractiveness of the para-pheromones for the different species. Generally speaking, methyl-eugenol (ME) is a stronger attractant than cue-lure (CL). Thus New Zealand uses a trap density of 1 per km^2 for ME trap and 8 per km^2 for CL traps. It is also probable that different species have a variable response to a given para-pheromone, though this has not yet been documented.

Other genera. For fruit fly not responding to para-pheromones, such as the *Anastrepha* spp., the use of Multilure traps (MLT) baited with Torula Yeast or '2C' (Amonium Acetate + Putrescine) may be recommended (Burnett *et al.*, 2006). Indeed, for the majority of *Anastrepha* spp. (i.e. *A. ludens*, *A. serpentina* and *A. obliqua*), '2C' appears more attractive than '3C' or Torula, and its use would allow the trap density to be decreased (Heath *et al.*, 2004; Burnett *et al.*, 2006; IAEA, 2006). However, '2C' and '3C' are known to be poorly attractive for *A. striata* and *A. fraterculus*, for which Torula Yeast remains the best attractant available (Burnett *et al.*, 2006; IAEA, 2006). In addition, this standard food attractant would also attract *Bactrocera* species not responding to ME and CL (Burnett *et al.*, 2006).

Rhagoletis species are surveyed in California with a mixture of ammonium acetate and protein hydrolysate baited on yellow panels (Pherocon AMTM traps). New Zealand uses a network of Nakagawa traps baited with hydrolysed proteins in addition to its specific networks for the general detection of all exotic fruit flies.

Whether liquid (protein baits, Torula Yeast) or solid ('2C', '3C') food attractants are used, MLT traps are currently recommended to replace other models of plastic McPhail traps such as the 'Dome trap' (IAEA, 2003). This should allow the density of traps to be decreased: compared with the use of 'Dome trap', 0.4 trap per km^2 can be deployed instead of 1.2–1.9 trap per km^2 for Mc Phail/Torula Yeast traps. In addition, for traps baited with liquid food attractants, the possible use of low-toxicity antifreeze instead of water would allow the servicing frequency to be decreased from 1 to 2 weeks. Such trapping systems based on food attractants attract the majority of tephritid species, including those whose males respond to a para-pheromone.

Pathways and commodities

The most common pathways consist of movement of travellers and the trade in fruit and vegetables. The first pathway is of considerable importance. In the USA, for instance, interceptions of pests and plants linked with passenger movements represented roughly 62% of the total interceptions during the period 1984–2000 (McCullough *et al.*, 2006). The pathways linked with human activity strongly influence the type of species found. For instance, pests intercepted in cargo or passenger baggage in Florida originated mainly from Central and South America, while the main origin was Australasia for California and Mexico for Texas (McCullough *et al.*, 2006). Texas and Arizona are the major gateways from Mexico to the USA for passengers and commercial shipments, respectively (Burnett *et al.*, 2006).

The complexes of fruit fly species that are present in surrounding countries, which may lead to infestations following natural dispersal, must also be taken into account. For

instance, *B. zonata*, which is present in very high populations in Egypt, may reach the European territory through natural dispersion as well as through movement of travellers.

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Aphids (Hemiptera: Aphididae)

(L. Marini)

Introduction

To date, 98 aphid species have been described which are present in Europe but originate from another continent. In addition, four cosmopolitan species of uncertain origin (cryptogenic species) have also been described. In comparison, the European aphid fauna currently includes 1373 species (Nieto Nafria, 2007), meaning that approximately 7.4% of the European aphid fauna is of alien origin (Cœur d'acier *et al.*, 2010). Only one species is currently included in the EU 2000/29 and the EPPO A2 list of pests recommended for regulation (*Toxoptera citricida*, the brown citrus aphid).

As they have mouthparts specially adapted for piercing and feed by sucking sap from plants, aphids often transmit plant viruses (e.g. to potatoes, cereals, sugarbeets and citrus plants). These viruses can sometimes kill the plants. They present mechanical, circulative and propagative virus transmission (Sylvester, 1980; van Emden & Harrington, 2007).

The brown citrus aphid *T. citricida* is specific to all citrus varieties. It infests the stems and new leaves of citrus trees and, in addition to causing feeding damage, it is a highly efficient vector of citrus tristeza virus (CTV). CTV can cause a range of symptoms in citrus trees, from mild and barely noticable to severe stem pitting and quick decline of trees, depending on the severity of the strain of

CTV and the susceptibility of the rootstock and scion combination. When sour orange is used as a rootstock, trees become very susceptible and then die from quick decline disease about 1–5 years after becoming infected. CTV can be detected by biological indexing and various non-biological methods, including light and electron microscopy, serology and a variety of molecular-based techniques. The latter include several types of reverse transcription polymerase chain reaction (RT-PCR), including an immunocapture PCR with multiple molecular markers (MMM; Hilf *et al.*, 2005) and real-time PCR (Ruiz-Ruiz *et al.*, 2009), SSCP analysis (Rubio *et al.*, 1996), oligoprobes (Narvaez *et al.*, 2000) and RFLP analysis (Gillings *et al.*, 1993). The following techniques are approved by the North American Plant Protection Organization (NAPPO, 2009): biological indexing, ELISA and immunoprint ELISA.

Detection and monitoring

Detection at borders is based mostly on direct visual inspection of host plants. Sticky traps and pan traps (a coloured cup filled with liquid) have both been used for monitoring flight activity of aphids, and are cheaper to buy than suction traps (van Emden & Harrington, 2007). However, sticky traps are attractive to many insects and must be replaced frequently. Furthermore, aphids caught in such traps require special solvents to remove them, and this process may make morphological identification difficult. Pan traps yield specimens in better condition, but also need to be emptied on a regular basis and are prone to flooding during periods of heavy rain. Furthermore, it should be kept in mind that traps monitor only the flight activity of alates; they provide little information on the survival or location of aphids in citrus crops and are not a substitute for effective survey techniques, i.e. physically searching crops for established colonies.

Suction traps have been used in entomology field research to sample a wide variety of insects. The rationale for using suction traps varies, ranging from basic documentation of what is moving into a particular area, monitoring insect vectors in association with crop plant epidemiological studies, to more advanced uses such as predictions of insect pest population densities. The latter is often possible only after many years of trapping coupled with field observations, detailed biological studies and population monitoring. The suction traps are approximately 8 m tall and sample flying aphids, with trap catch representative of flight activity within a 50 km radius.

Monitoring efforts should be focused on citrus plants. Brown citrus aphid species can be differentiated from other aphids infesting citrus based on body colour (brown). This species inhabits stems as well as the leaves of citrus, and when squashed it produces a reddish brown colour. In countries where *T. citricida* occurs, groves should be inspected throughout the year, as should any plant material being transported as well as plants for planting. Particular attention should be paid to the young growth. When aphids are scarce, the undersides of mature foliage should be examined for dead or parasitized aphids or mummies, which adhere to the leaves and can be used for identification in the absence of living specimens. If the aphid cannot be positively identified on the spot using a pocket lens, specimens should be preserved in 2:1, v/v, 95% ethanol : 75% w/w lactic acid, or kept alive in small tubes and taken to a laboratory for confirmation using microscopy.

Alates are not strong fliers and few fly far from their parent colony (Gottwald *et al.*, 1995). Gavarra & Eastop (1976) obtained better catches of *T. citricida* in yellow Moericke trays at 152 cm height than they did in trays at ground level. Consequently, the optimal placement of traps is thought to be above ground level, but lower than the height of surrounding trees. Lara *et al.* (1976) used water traps to compare the attractiveness of various colours to a number of different insects attacking citrus. In general, they found yellow and white to be the most attractive to all species, including the aphid and its predators, the coccinellid *Cycloneda sanguinea* and the lacewing *Chrysopa* sp. However, Schwarz (1965) found that the relative attractiveness of yellow and green to *T. citricida* changed seasonally, and varied from year to year.

Pathways and commodities

Most aphids travel long distances with air currents so most species are cosmopolitan and are not included in quarantine lists. The pathways for introduction are known only in a very small number of cases. Most Aphididae have a high level of host-plant specificity and most alien species are therefore thought to have been introduced into Europe with their host plants.

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Leafhoppers, planthoppers (Hemiptera: Clipeorrhyncha, Archeorrhyncha) and psyllids (Hemiptera: Psyllidae)

(N. Mori)

Introduction

Many leafhoppers and planthoppers, and some psyllids, are important pests of crop plants, particularly because they are vectors of virus, bacteria and phytoplasma diseases. Around 200 species are already known to spread plant disease, but many more are likely to be recognized (Wilson & Turner, 2010).

Several vector species that belong to the order Hemiptera are currently included in the Council Directive 2000/29 and EPPO lists, among which four Cicadellidae: *Draeculacephala minerva* (grass sharpshooter), *Graphocephala atropunctata* (blue-green sharpshooter), *Homalodisca coagulata* or *vitripennis* (glassy-winged sharpshooter), *Scaphoideus luteolus* (white-banded elm leafhopper), and two Psyllidae: *Diaphorina citri* and *Trioza erytreae* (citrus psyllids).

In Europe the most important diseases transmitted by these insects involve phytoplasmas, bacteria, viruses and viroids. Within the order Hemiptera, the family Cicadellidae contains most of the phytoplasma vector species, followed by the families Cixiidae, Delphacidae, Derbidae, Cercopidae and Flatidae in the superfamily Fulgoroidea. Finally, two genera in the family Psyllidae also include species that can transmit important phytoplasmas to fruit trees (Weintraub & Beanland, 2006). On apple trees, Candidatus *Phytoplasma mali* (Apple proliferation) is transmitted by *Cacopsylla melanoneura* (Forster; Tedeschi *et al.*, 2002), *C. picta* (=*costalis*; Frisinghelli *et al.*, 2000) and *Fieberiella florii* (Krczal *et al.*, 1988; Tedeschi & Alma, 2006); on pear trees, Candidatus *Phytoplasma pyri* (Pear decline) is transmitted by *C. pyri* (Carraro *et al.*, 1998a) and *C. pyrisuga* (Grbic, 1974). On stone fruit, Candidatus *Phytoplasma pruni* (European stone fruit yellows) is transmitted by *Cacopsylla pruni* (Carraro *et al.*, 1998b) and on vitis the grapevine yellows Candidatus *Phytoplasma vitis* (Grapevine flavescence dorée) and Candidatus *Phytoplasma solani* (Stolbur) are respectively transmitted by *Scaphoideus titanus* (=*littoralis*; Schvester *et al.*, 1963) and *Hyalesthes obsoletus* (Maixner, 1994; Sforza *et al.*, 1998; Alma *et al.*, 2002; Mori *et al.*, 2002; Bressan *et al.*, 2007).

Regarding the vector species included in the EU 2000/29 and EPPO lists, *D. minerva*, *G. atropunctata*, and *H. coagulata* transmit grapevine Pierce's disease (*Xylella fastidiosa*; Purcell & Saunders, 1999; Cabrera La Rosa *et al*., 2008), *S. luteolus* transmitted American Elm yellows/16SrV-A (Baker, 1948), *D. citri* and *T. erytreae* transmit Candidatus *Liberibacter* spp. (Citrus greening disease; McClean & Oberholzer, 1965; Catling, 1970).

Detection and monitoring

Typically, when new phytoplasma diseases are discovered, little is known about the disease epidemiology. The first step in identifying the species of insects that transmit the agent to the crops is to determine which insects are found in the vicinity of the diseased plants (Weintraub & Beanland, 2006). Season-long monitoring must be conducted. The most common methods to monitor the insect population in and near crops is by the use of yellow sticky traps (Weintraub & Orenstein, 2004), sweep netting (Pilkington *et al.*, 2004), vacuum sampling (Weintraub & Orenstein, 2004), or shaking branches over collection trays to capture falling insects (Carraro *et al.*, 2004). The latter methods are effective survey techniques that yield live insects. Another technique useful in phytoplasma vector studies is based on the use of a Malaise trap, which can capture flying insects which are not captured by other trapping methods. In addition, when placed at the interface of two habitats, bidirectional Malaise traps allow the determination of net movement patterns of trapped insects (Irwin *et al.*, 2000) or movement between forest vegetation and crops (Altieri & Schmidt, 1986).

Pathways and commodities

Plants for planting is the major pathway, while various human activities contribute to spread (Bertin *et al.*, 2007).

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Whiteflies (Homoptera: Aleyrodoidea)

(W.J. De Kogel)

Introduction

Whiteflies are polyphagous pests that occur in a wide range of ornamental and vegetable crops (Byrne & Bellows, 1991; Oliveira *et al*., 2001). Well known pest species in Europe are *Trialeurodes vaporariorum* and *Bemisia tabaci.* Apart from direct feeding damage to plants, whiteflies transmit a large number of plant viruses (Jones, 2003).

Bemisia tabaci whiteflies are present in Europe, but the non-European populations are in Annex IAI of the EC Plant Health Directive 2000/29. *Bemisia tabaci* whiteflies are of interest from a quarantine point of view principally due to the quarantine viruses they can transmit. Whiteflies feed on the phloem of plants, and during feeding they can take up and transmit plant viruses. Examples of such viruses are (a) Bean golden mosaic virus, (b) Cowpea mild mottle virus, (c) Lettuce infectious yellows virus, (d) Pepper mild tigré virus, (e) Squash leaf curl virus, (f) Euphorbia mosaic virus, (g) Florida tomato virus. Molecular tools are being developed to test for the presence of such viruses in the insect.

Detection and monitoring

Whiteflies can be detected by plant sampling as both adult and immature stages are present on the above-ground plant parts. Alternatively, yellow sticky traps are being used as the yellow colour is attractive to the adult flies. There are a few reports suggesting that attractive odours could improve trapping efficacy (Li & Maschwitz, 1983; Baranowski & Blaszak, 1996; Gorski, 2003). Once whiteflies have been intercepted, identification to species (and biotype) level is based on either morphological characteristics or molecular markers (Bosco *et al.*, 2006; Papayiannis *et al.*, 2009).

In the EU, efforts are now being made to develop molecular tools for detection of the viruses present in whitefly specimens. In Europe, import of plant material from outside the EU that contains *B. tabaci* is not allowed, and action is

taken when *B. tabaci* is detected. The objective is to prevent the entry of non-European populations of *B. tabaci* that may contain quarantine viruses.

Pathways and commodities

Whiteflies are associated with a wide range of ornamental and vegetable crops. They are imported into the EU by shipments of host plants.

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Moths and butterflies (Lepidoptera)

(D.C. Lees and S. Augustin)

Introduction

To date, seven invasive alien lepidopteran species have been recorded in Europe (Lopez-Vaamonde *et al.*, 2010) and 33 moth/butterfly species are currently listed as quarantine species (11 in the EU Council Directive 2000/29, 15 in EPPO list A1 – absent from the EPPO region, 16 in list A2 – locally present in the EPPO region). These pests attack crops; forest, ornamental or fruit trees; and stored grain. These 33 species comprise 10 Tortricidae, 7 Noctuidae, 5 Lasiocampidae, 3 Pyralidae, 2 Lymantriidae, 2 Gelechiidae, 1 Castniidae, 1 Tineidae, 1 Carposinidae and 1 Lycaenidae. Several of these species, the most recent being *Tuta absoluta*, have established and spread in Europe only in the past few years and now pose a serious threat to agricultural and horticultural crops (Desneux *et al.*, 2010). Where relevant to discussion of methods, a few extra potential quarantine species are mentioned that are described in the chapter of Lopez-Vaamonde *et al.* (2010), such as *Cameraria ohridella*, which originates in the Balkans. The detection methods (visual detection, light, pheromone and synergistic traps) and trapping systems that are useful for the detection and monitoring of particular species are briefly described.

Detection and monitoring

Quarantine species of Lepidoptera are usually detected as flying adults by light or by pheromone traps. Sometimes larvae or emerging females can be trapped with sticky bands around tree trunks.

For monitoring and early detection, Malaise traps are sometimes used, although, due to loss of scales when alcohol is used, these are less useful for rapid identification of Lepidoptera than for other orders of insects. Like Malaise traps, suction traps are not very practical for sorting and identifying samples. Light traps (the Rothamsted trap, the Robinson Mercury Vapour trap, and many other types of ultraviolet light trap) are a more efficient general method for collecting nocturnal insects (see supporting information http://archives.eppo.int/files/pratique_42_1/augustin.xls); however, *Cacyreus marshalli* and *Paysandisia archon* are diurnal and do not use pheromones (Sarto I Monteys *et al*., 2012). Sometimes, as in the case of the Rothamsted trap design, tetrachloroethylene (Perchlor) is used as an automatic killing method (other insecticides are used in several proprietary 'attract and kill' systems). The advantage of the Rothamsted type of trap is standardization for geographical monitoring of presence and yearly abundance on a grid. Pheromones can also be combined with emission of light at a wavelength attractive to adults, such as the commercially available Ferolite system (Russell IPM) for mass trapping of *Tuta absoluta*. For monitoring for a particular pest, it is advisable to use a combined 'monitoring and mass trapping' system.

Pheromones, where commercially available, are extremely useful for detection of forest Lepidoptera (Grant, 1991), as well as for invasive species in urban or suburban areas (Augustin *et al.*, 2004). They are also the most promising method for mass trapping and 'lure and kill' solutions and are used successfully for some lepidoptera (El-Sayed *et al.*, 2006, 2009). Specialized pheromones are commercially available for at least 15 of the quarantine species (supporting information available on http://archives.eppo.int/files/pratique_42_1/augustin.xls).

If the source references for the pheromones are required, the user can look them up in the Pherobase database (www.pherobase.com). A range of pheromone traps are available. A similar design and sometimes the same pheromone blend can be used for several species. Sticky glue traps are often used for trapping, although this can sometimes make it difficult to identify the sample.

For a number of species, useful advice is given online, and proprietary traps from a number of suppliers are available. For example, for *Tecia solanivora*, *Leucinodes orbonalis* and *Helicoverpa armigera*, the Delta and Moth

Catcher traps (Russell IPM) and pheromone traps are available, with advice on handling (www.russellipm.com).

As part of an integrated pest management (IPM) strategy, use of pheromones in mass mating disruption may be a viable strategy, especially in crop situations, for some Lepidoptera (Kyparissoudas, 1989; Nicholas *et al.*, 1999; Walker & Welter, 2001), usually at low to intermediate population density with 1–2 traps per hectare (Welter *et al.*, 2005). In the case of *Grapholita molesta* in peach orchards, a range of hand-applied (laminate, membrane or rope), puffer-type and sprayable microencapsulation dispensing methods have been evaluated (Pickel *et al.*, 2002), which include high-emission devices (Shorey & Gerber, 1996), and should work well for many similar species e.g. *Choristoneura rosaceana* (Welter *et al.*, 2005).

Parapheromones, artificially synthesized compounds that inhibit pheromone detection or disrupt communication in Lepidoptera (Renou & Guerrero, 2000), are used for *Choristoneura occidentalis* (McLean *et al*., 1989) and may be a future avenue to pursue, where the available pheromones are unstable or unavailable for key species. Kairomones could also be used for detection of some moths, e.g. floral traps for monitoring female *H. armigera* populations (Bruce *et al.*, 2002).

Pheromone traps or synergistic traps seem to be best for Lepidoptera. Where lures are not available, traditional trapping and detection methods should be used (see supporting information at http://archives.eppo.int/files/pratique_42_1/augustin.xls).

Pathways and commodities

The main pathway for invasion of Lepidoptera is via plants transported by the horticultural industry. A typical example is *P. archon* imported with palms (Lopez-Vaamonde & Lees, 2010); unfortunately detection and monitoring methods for this species are essentially visual. A few species (mainly Gelechiidae) are transported with grains and seeds (Lopez-Vaamonde *et al.*, 2010). International transport of fruits via containers and packaging equipment (e.g. crates) and vehicles is also an important pathway: e.g. tomato with *T. absoluta* or potato tubers with *Tecia solanivora*. In many cases, as on arrival of commodities or before transport, early instars, mainly larvae or their mines, need to be detected visually on plants that are transported by the horticultural industry. In some cases, early stages are cryptic and visual spotting, e.g. of meristem damage, is a vital step in phytosanitary inspection and monitoring, as for the lycaenid geranium bronze, *Cacyreus marshalli* (De Prins & De Prins, 2010). Moth migration, e.g. in the genus *Spodoptera*, can be considered a complementary pathway.

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Thrips (Thysanoptera)

(W.J. De Kogel)

Introduction

Thrips, including *Frankliniella occidentalis*, *Thrips tabaci* and *T. palmi* (Thysanoptera: Thripidae), are key pests of greenhouses and outdoor crops (Lewis, 1997; Kirk & Terry, 2003; Cannon *et al.*, 2007) because of their ability to damage plants directly through feeding, and indirectly through transmission of plant viruses (Jones, 2005). Biological attributes such as polyphagy, vagility, rapid reproduction, cryptic behaviour and insecticide resistance make them particularly difficult to manage (Mound & Teulon, 1995; Morse & Hoddle, 2006). In Europe, *Thrips palmi* is listed as a major quarantine pest. Thrips are well known vectors of a number of plant viruses, especially tospoviruses such as Tomato spotted wilt virus (TSWV; Jones, 2005; Pappu *et al.*, 2009).

Detection and monitoring

Detection of thrips can be done by plant sampling, since both adults and immature stages are present on the above-ground parts of the plant. Alternatively, blue (or yellow) sticky traps or water traps are used. The colour is attractive to the adult insects and will attract adults over a short distance. There are a number of reports showing that attractive odours, such as pheromones (De Kogel & van Deventer, 2003; Kirk & Hamilton, 2004; Hamilton *et al.*, 2005) or plant odours and derivatives (Kirk, 1985; Teulon *et al.*, 1993; Murai *et al.*, 2000; Teulon *et al.*, 2007; Davidson *et al.*, 2008), can improve trapping efficacy. Once thrips are intercepted, identification to the species level is based on either morphological characteristics (Moritz *et al.*, 2004) or molecular markers (Brunner *et al.*, 2002; Glover *et al.*, 2010). EPPO provides standard diagnostic protocols for this purpose (http://archives.eppo.int/EPPOStandards/diagnostics.htm).

Monitoring of thrips is done mainly by using blue sticky traps (or water traps) and by looking at symptoms. Typical thrips symptoms are silvery feeding scars on leaves and fruits of plants. Heavy infestations may lead to scars, stunting and malformation of shoots and fruits.

Thrips, including *T. palmi*, are difficult to control chemically in the field and especially in glasshouses. Insecticides such as imidacloprid and pyrethroids are used, but may have serious undesirable effects on natural enemies (Nemoto, 1995).

Pathways and commodities

Most thrips species have a broad host plant range and can be associated with many different ornamental, vegetable and fruit crops. They have limited natural spread, but can be transported over long distances with plant material.

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Surveillance techniques for exotic pathogens

(N. Boonham)

Introduction

Surveillance for exotic pathogens is usually performed by inspection services where the front line is the finding and identification of disease symptoms by visual means. In most cases this initial visual inspection is followed up in the laboratory using diagnostic or identification techniques. The diagnosis and identification of plant pathogens currently relies on a very diverse range of techniques and skills, from traditional culturing and taxonomic skills to modern molecular-based methods (Boonham *et al.*, 2008). The wide range of methods employed reflects the great diversity of plant pathogens and the hosts they infect. The well documented decline in taxonomic expertise, along with the need to develop ever more rapid and sensitive diagnostic methods, has provided an impetus to develop technologies that are both generic and able to complement traditional skills and techniques. Real-time polymerase chain reaction (real-time PCR or Q-PCR) is emerging as one such generic platform technology and one that is well suited to high-throughput detection of a limited number of known target pathogens. Real-time PCR is now exploited as a frontline diagnostic screening tool in human health, animal health and homeland security, as well as plant health. Progress with developing generic techniques for plant pathogen identification, particularly of unknown pathogens, has been less rapid. Diagnostic microarrays and direct nucleic acid sequencing (*de novo* sequencing) both have potential as generic methods for the identification of unknown plant pathogens, but are unlikely to be suitable as high-throughput methods in their current formats.

More recently, detection (as opposed to diagnostic and identification) methods are being developed for pathogens: these methods are more akin to surveillance tools that seek to locate infected material such that more specific detailed work can be performed. These methods may be characterized by being highly sensitive in the first instance, while specificity can be achieved by follow-on confirmatory testing.

Viruses

Surveillance for viral pathogens is performed almost always by visual means, either by direct observation of symptoms in the field or in produce, or potentially remotely such as by examination of aerial photographs and patterns of symptoms (reviewed in Bock & Nutter, 2011). In common with other detection methods, visual examination tends to have a low diagnostic specificity in terms of identifying the pathogen, but is highly useful in directing sampling efforts and in the surveillance of large areas, prior to follow-on testing using a more specific laboratory method.

Plant viruses are an inherently diverse group that, unlike cellular pathogens, possess no conserved genes (e.g. ribosomal RNA sequences) that are common to all viruses (Boonham *et al.*, 2007). Detection of plant viruses is becoming more challenging as globalization of trade, particularly in ornamentals, and the potential effects of climate change enhance the movement of viruses and their vectors, transforming the diagnostic landscape. As a result, surveillance for viruses in a laboratory setting will usually tend towards methods that can be performed in high-throughput for testing large numbers of samples for the presence of the target virus (e.g. using ELISA or real-time PCR methods). Other techniques that can be used for surveillance of pathogens in material such as seed, other propagation materials and field samples are those that can be used broadly (often refered to as multiplex or universal methods) for the detection of viruses in many different groups, including biological indexing, electron microscopy, microarray methods and, more recently, next generation sequencing. Of these, methods based on microarray detection (reviewed in Boonham *et al.*, 2007) and next generation sequencing (Studholme *et al.*, 2011) provide the greatest capability for parallel yet specific testing, and can be used to detect individual viruses or combinations of viruses.

Lateral flow devices

Lateral flow devices (LFDs) are rapid, inexpensive, disposable and simple to use, and as a result can be used in the field to detect pathogens. Although used mostly to confirm the identification of a pathogen from symptomatic material, the speed and cost mean they could be deployed for surveillance, though for non-symptomatic material it is likely to be impractical to test enough samples in the field. A positive test result is signified by the appearance of a line at the 'test' position within 10 min of the addition of a sample to the device, making the results easily interpretable by a non-specialist. LFDs are available for *Phytophthora* (Lane *et al.*, 2007) that can be used to confirm the presence of *Phytophthora* in symptomatic plant material. The serological reagents available for use within these devices are not species-specific, so samples that are positive for *Phytophthora* are returned to the laboratory for confirmatory testing for *P. ramorum* and *P. kernoviae* using species-specific molecular tools. The *Phytophthora* LFDs have been found to have high diagnostic sensitivity when compared with species-specific methods (both PCR-based and cultural methods), indicating their suitability for use as a pre-screening method in the field (Kox *et al.*, 2007;

Lane *et al.*, 2007). These devices also compared favourably with *Phytophthora* spp. ELISA in terms of diagnostic specificity (Kox *et al.*, 2007). However, the use of LFDs can be limited for some pathogens where the target is present in a low titre or out of range of a serological test, or where serological reagents are not available with the required specificity (e.g. they are unable to detect all strains of a pathogen or to discriminate between closely related species). In a laboratory situation, these problems are usually resolved by using nucleic acid-based techniques such as PCR. These techniques are discussed in the following section on case studies of exotic pathogens, as they have a general interest.

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Specific groups of exotic pathogens

(A. Yart)

Bacteria, potato brown rot (*Ralstonia solanacearum*)

Identity: Bacteria, Gracilicutes, Proteobacteria.
Synonyms: *Pseudomonas solanacearum* (Smith), *Burkholderia solanacearum* (Smith) (Yabuuchi *et al.*, 1992)

Introduction

Ralstonia solanacearum causes bacterial wilt on many solanaceous cultivated plants and is a quarantine pathogen classified in EU Annex designation I/A2 and in the EPPO A2 list. It is a complex bacterial species divided into races, biovars (Fegan & Prior, 2005), each one being adapted to various climatic zones. Its host range is quite wide (over 200 plant species; Lyons *et al.*, 2001) and includes woody plants (Supriadi *et al.*, 2001). In temperate areas it mainly attacks potato and tomato plants, and can also be found on solanaceous weeds such as *Solanum nigrum* and *Solanum dulcamara*.

EPPO organized a conference on *Ralstonia solanacearum* (EPPO, 1997) and has already published a *Ralstonia solanacearum* diagnostic protocol (EPPO, 2004a) with a list of detection and identification methods, as well as control systems (EPPO, 2004b), early detection methods being essential for surveillance. As is the case for many bacterial infections, *R. solanacearum* can spread very easily with infected plants, contaminated soil and water used for irrigation. Even water courses may be contaminated with infected solanaceous weeds (Elphinstone *et al.*, 1997). *Stenotrophomonas maltophilia* seems to have an antagonistic potential against *R. solanacearum* (Messiha *et al.*, 2007) and could be used as a biocontrol agent.

Detection and monitoring

Bacterial infections may be introduced with plants, e.g. imported seed potatoes, without any external symptoms. Reliable and rapid detection techniques which can be used *in situ* would greatly enhance the application of surveillance and control programmes.

In Portugal, a *Staphylococcus aureus* slide agglutination test was used directly on tomato and potato plants in the field, and on bacterial cultures under laboratory conditions (Lyons *et al.*, 2001). Stefani *et al.* (2005) describe a non-destructive analysis protocol to detect and survey latent infections in tomato plants, combining a selective medium, PCR and IFAS.

Laboratory detection methods are becoming increasingly sensitive. Grover *et al.* (2009) used MDA-PCR to detect ultra-low populations; Smith and de Boer (2009) developed an improved TaqMan method for PCR; Poussier *et al.* (2005), Alvarez Restrepo *et al.* (2008) and Lin *et al.* (2009) used a BIO-PCR protocol that can be selective and rapid (Paret *et al.*, 2008; Kutin *et al.*, 2009); Kubota *et al.* (2008) developed a loop-mediated amplification of DNA (LAMP).

Pyruvate-amended selective medium was used to improve the detection sensitivity of *R. solanacearum* (Imazaki & Nakaho, 2010). Using the post-enrichment DAS-ELISA technique, the detection of bacterial wilt latent infection in potato stem pieces about 3 weeks before harvest has been shown to be reliable and simple to apply (Priou *et al.*, 2010). It can also be used for soil samples (Priou *et al.*, 2006).

Kawasaki *et al.* (2007) propose an easy-to-use GFP-tagging tool for any strain of *R. solanacearum* in laboratory and field studies. Monoclonal antibodies may be used for specific detection in soil (Farida *et al.*, 2007) or in water (Biosca *et al.*, 2005), but real-time PCR combined with a protocol to

extract DNA from soil provides an important tool for routine detection in soil samples (Huang *et al.*, 2009).

Bioindicators for *R. solanacearum* have also been tested (Paret *et al.*, 2009). Monitoring based on PCR methods has been used to analyse the pathogen's distribution in soil and water (Reza *et al.*, 2008). Depending on races and biovars classified by RFLP studies, *R. solanacearum* has been found in tropical areas all over the world as well as in temperate areas (EPPO, 2004a). The lower temperature optimum for race 3 may explain the establishment of potato brown rot in Belgium, France, Germany, Hungary, Italy, Netherlands, Portugal, Spain, Slovakia and the UK (Stefani *et al.*, 2005).

In the UK, detection at one site containing ware potatoes was followed by phytosanitary measures to eradicate the disease (EPPO, 2010a). A detailed report about monitoring and control of the potato brown rot bacterium in UK has been published (Elphinstone, 2001).

In Italy (Sardinia), a survey was conducted in glasshouses where tomato plants showing bacterial wilt symptoms were observed. Identification was confirmed using semi-selective media, bioassays on tobacco leaves, immunofluorescence tests and PCR, and several eradication measures were carried out: uprooting and burning of plants, methyl bromide treatment and decontamination of stored water with a sodium chloride solution (Fiori *et al.*, 2009; EPPO, 2010b).

In Portugal (EPPO, 2010c), *R. solanacearum* race 3 biovar 2 reappeared in potato and tomato fields (including solanaceous weeds and irrigation water) in the late 1990s. In 2007, potato plants showing symptoms were tested (isolation on semi-selective medium, IF, PCR, sequencing, bioassays) and *R. solanacearum* biovar 1 was detected for the first time in Portugal. Specific phytosanitary measures were taken.

Pathways and commodities

In European countries, stored seed and ware potatoes, potato and tomato plants are the main inspected commodities. Geranium and other plants from nurseries are also inspected (Janse *et al.*, 2009). In Sweden and the UK, infected ware potatoes were found and probably originated from infected seed potatoes from Netherlands (EPPO, 2010a). In Italy (Sardinia), the origin of the introduction and spread of infection in tomato plants cultivated in glasshouses is unknown (Fiori *et al.*, 2009).

Genetic studies in the Republic of Korea suggest the international trade of potatoes spreads this pest (EPPO, 2007).

The detection of *R. solanacearum* race 3 biovar 2 in *Pelargonium* plants in Netherlands, Germany, England and Belgium in 2002 was traced to contaminated irrigation water used during plant production in Kenya (Janse *et al.*, 2005, 2009).

Race 1 may be introduced with ornamental plants of tropical origin grown in glasshouses in temperate climates (EPPO, 2004a).

Generalization to other plant pathogenic bacteria

Ralstonia is probably the most destructive plant bacterium worldwide, but test methods have been standardized and validated for other economically important quarantine bacteria (Janse, 2005).

A DNA extraction minikit (to ensure the detection by PCR) was also used for other bacteria (Poussier *et al.*, 2005). The real-time PCR method is increasingly used for detection of other plant bacteria (e.g. *Clavibacter michiganensis*, Seigner *et al.*, 2007; Zaccardelli *et al.*, 2010), but an integrated approach (isolation, serological tests, PCR and bioassays) would allow more accurate detection of *Erwinia amylovora*, *Ralstonia solanacearum and Xanthomonas axonopodis* pv. *citri* in plant material (Lopez *et al.*, 2005).

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Fungi, pitch canker disease (*Gibberella circinata*)

(A. Yart)

Identity: Fungi, Ascomycota, Hypocreales, Nectriaceae.
Teleomorph: *Gibberella circinata* Nirenberg & O'Donnell
Anamorph: *Fusarium circinatum* Nirenberg & O'Donnell
Synonyms: *Fusarium subglutinans* f. sp. *pini* Hepting, *Fusarium moniliforme* Sheldon var. *subglutinans* Wollenweber, *Fusarium lateritium* f. sp. *pini* Hepting.

Introduction

Gibberella circinata is the causal agent of pitch canker disease, which may affect all the *Pinus* species but also Douglas fir (*Pseudotsuga menziesii*; Gordon *et al.*, 2006; Anonymous, 2009). This disease, which causes cankers

that girdle branches, is a serious threat to pine forests because tree mortality may occur after multiple branch infection. Pitch canker is a significant threat to countries where non-native and susceptible *Pinus* spp. are grown intensively in plantations (Kim *et al.*, 2008; Wingfield *et al.*, 2008). Moreover, *Gibberella circinata* may also be soil-borne, and may cause root rot even in mature trees (Garbelotto *et al.*, 2007) and infect seeds externally or internally (without any symptoms before seed germination).

The anamorph, *Fusarium circinatum*, is a wound pathogen and may occur following mechanical wounding (Sakamoto & Gordon, 2006) and following woodboring insect damage (Anonymous, 2009). Tree infection occurs by aerial dispersion of conidiospores or through transmission by feeding insects (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004).

Climate-based models have been developed to predict global risk of pitch canker establishment (Ganley *et al.*, 2009). The management and control of this disease are dependent on the accurate and timely diagnosis of the pathogen (de Wet *et al.*, 2010). The contribution of molecular analysis is important (Garbelotto, 2008).

In 2000 EPPO published a Pest Risk Assessment (PRA) report about this pathogen. This pest is now in the EPPO A1 list (no. 306) as a quarantine pest recommended for regulation and a diagnostic protocol has recently been published (EPPO, 2009d).

In Northern Spain, a study on 11 bark beetle species (Coleoptera: Scolytinae) and one root weevil (Coleoptera: Entiminae) showed their association with *Fusarium circinatum.* Bioassays using funnel traps with verbenone were performed to test a possible integrated management strategy (Romon *et al.*, 2007). Fox *et al.* (1991) previously demonstrated that *Ips mexicanus* and *Ips paraconfusus* could transmit pitch canker disease to *Pinus radiata*. The twig bark beetles *Pityophthorus setosus* and *Pityophthorus carmeli* are known as *Fusarium circinatum* vectors in California, where wounded healthy branches become suitable for infection (Sakamoto *et al.*, 2007).

Erbilgin *et al.* (2009) suggested that an initial infection by these beetles (*Pityophthorus setosus* and *Pityophthorus carmeli*) may induce resistance to subsequent infections of the host. *Nemosoma attenuatum* (Coleoptera: Trogossitidae), associated with twig beetles in the genus *Pityophthorus*, is also a potential vector of *Fusarium circinatum* (Sakamoto, 2007).

Beetle species associated with *Fusarium circinatum*-infected Monterey pines in California (*Ips mexicanus* and *Ips plastographus*) may indicate a higher risk of pitch canker transmission depending on the level of infection and the propagule load in spring (Erbilgin *et al.*, 2008).

Conophthorus radiatae (Coleoptera, Scolytidae), *Ernobius punctulatus* (Coleoptera, Anobiidae) and *Pityophthorus* spp., which infest *Pinus radiata*, as well as *Cephalonomia utahensis* (Hymenoptera: Bethylidae), a parasitoid of *Ernobius punctulatus,* represent another potential source of inoculum (Hoover *et al.*, 1995, 1996).

Among fungi associated with phloeophagous insects colonizing *Pinus radiata*, two non-pathogenic fungi, *Fusarium lateritium* and *Penicillium chrysogenum*, have potential biocontrol abilities for *Fusarium circinatum* (Romon *et al.*, 2008).

Detection and monitoring

As for many other fungal diseases, visual inspection, symptomatic plant tissue sampling and isolation on semi-selective medium are the first steps for pathogen detection and identification.

The composition of several culture media that are adapted to isolate and identify any *Fusarium* spp. using morphological criteria have been described (EPPO, 2009a–d). However, to ensure correct *Fusarium circinatum* identification, molecular methods are often required.

A PCR-RFLP test was developed for use only with pure *Fusarium circinatum* cultures (Steenkamp *et al.*, 1999). A detailed characterization of pitch canker fungus isolates using PCR-RFLP analysis was used to confirm the establishment of the pathogen in Chile (Jacobs *et al.*, 2007) and in Spain (Perez-Sierra *et al.*, 2007), where mating types showing virulence differences were identified by multiplex PCR. As pitch canker could be a threat for the New Zealand forest industry, a PCR-based diagnostic method was developed to detect the pathogen within infected host tissues and in infested soil (Ramsfield *et al.*, 2008).

In order to develop a fast and reliable diagnostic test independently of the presence of disease symptoms, Schweigkofler *et al.* (2004) present a novel trapping approach using filter paper in combination with a rapid molecular method to detect the presence of inoculum and to quantify it in the air. The test can be used directly on trapped spores, without the need for spores to be germinated.

Compared with more traditional approaches, SYBR-green real-time PCR allows identification with increased sensitivity and higher selectivity independently of the presence of symptoms (Schweigkofler *et al.*, 2004).

Recently, a new detection protocol based on a biological enrichment step followed by a real-time PCR assay was developed in order to allow a quick and reliable detection of *Fusarium circinatum* in pine seeds (Ioos *et al.*, 2009). A recent study confirmed IGS PCR-based diagnostic procedures specificity (de Wet *et al.*, 2010).

Gibberella circinata, officially reported in USA, Mexico, Haiti, South Africa, Japan, Republic of Korea and Chile (EPPO 2005), has been reported recently in Europe, but infested areas remain restricted (EPPO 2009d). Pitch canker was described in several regions in Spain, but these were always isolated outbreaks originating from nurseries (EPPO, 2005, 2006a). It was first reported in France in 2006 on declining pines and Douglas fir (EPPO, 2006b), and visual

inspection combined with laboratory tests confirmed pest eradication (EPPO, 2008). But new isolated outbreaks (Vosges 2008 and Vendée, Côtes d'Armor; EPPO, 2009a) have been reported, and studies were initiated to identify the origin of the infection (EPPO, 2009a, 2010). During the same period, *Gibberella circinata* dieback symptoms in Italy were identified using morphological and cultural characteristics confirmed by PCR with specific primers (Carlucci *et al.*, 2007), and the fungus was eradicated (EPPO, 2009b). In Portugal its presence on symptomatic plant samples was confirmed by PCR and pathogenicity tests after a first identification based on morphological and cultural characteristics (EPPO, 2009c; Bragança *et al.*, 2009).

As outbreaks are isolated and far away from each other, it is important to monitor the origin of the infection: in France, studies were carried out on conifer seeds imported from the USA, and infected seed lots and seedlings from these lots were detected and destroyed. A buffer zone was demarcated around the infested site and subjected to intensive monitoring (EPPO, 2009a). When infected pines were detected in a nursery, eradication measures (plant destruction and increased monitoring) were carried out immediately and the information was provided to all the customers of the nursery (EPPO, 2010).

The spore trapping method combined with real-time PCR (Schweigkofler *et al.*, 2004) was used to evaluate aerial dispersal, providing important epidemiological information (Garbelotto *et al.*, 2008). Assaying pine cones for surface contamination may be useful in monitoring the inoculum level in geographical locations (Dwinell, 1998).

Pathways and commodities

Although the fungus may be introduced into Europe by several pathways (seedlings, wood, insect vectors), the most important risk of introduction is by seed (EPPO, 2000). An exhaustive study of seed driers, nurseries and established plantations, which investigated seeds, nursery seedlings, wood with resinous cankers, flowers and cones of *Pinus* spp. throughout Galicia, was carried out in Spain when *Fusarium circinatum* was detected (Gonzalez Penalta *et al.*, 2008). In France, the National Plant Protection Organization carried out studies on conifer seeds imported from the USA and detected *F. circinatum* in six seed lots of *Pinus taeda*, *Pinus ponderosa* and *Pseudotsuga menziesii* (EPPO, 2009a).

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Fungi, brown rot disease (*Monilinia fructicola)*

(A. Yart)

Identity: Fungi, Ascomycota, Helotiales.
Teleomorph: *Monilinia fructicola* (Winter) Honey
Anamorph: *Monilia fructicola* Batra
Synonyms: *Sclerotinia fructicola* (Winter) Rehm

Introduction

Monilinia fructicola causes brown rot mainly on stone fruits (*Prunus* spp.) and may also affect other rosaceous fruit trees (*Malus* and *Pyrus*; Sholberg *et al.*, 2003). The disease may destroy a crop by killing blossoms, or by rotting mature fruits on the tree, or after harvest (EPPO, 2009b). The anamorph (*Monilia fructicola*) may also cause brown rot. *Monilinia fructicola* does not depend on specific vectors for propagule dispersal; conidia may be dispersed by wind, water and many kinds of vector, e.g. insects and birds (Van Leeuwen *et al.*, 2001)

Two other *Monilinia* species (*Monilinia fructigena* and *Monilinia laxa*) causing brown rot, which have been present in Europe for a long time, must be distinguished from *Monilinia fructicola* (Hughes *et al.*, 2000; Ioos & Frey, 2000; Lane 2002), the spread of which in Europe would significantly increase crop loss, especially in peach, nectarine and apricot.

Already established in North and South America, Japan and Australia (EPPO/CABI, 1997), this pathogen was introduced in Europe (first report in France in 2001). A recent distribution map summarizes the current situation (CABI, 2010). Geographical distribution records of pathogens and pests are the basis for phytosanitary decision-making. Molecular techniques with species-specific primers for *M. fructicola*, *M. laxa* and *M. fructigena*, based on the EPPO Diagnostic Protocol for *M. fructicola*, were used for the identification of presumed positive *Monilinia* isolates in South Africa. The regulated status of *M. fructicola* in South Africa was justified scientifically by the results from this survey (Carstens *et al.*, 2010). Very large areas of stone fruit cultivation are found in Southern Europe, where climatological conditions are suitable for *M. fructicola* establishment.

A European survey was undertaken after the detection of *M. fructicola* in several European countries. Strict import regulations have been adopted by the EU (Van Leeuwen *et al.*, 2001). First reported in France (EPPO, 2002a), where prophylactic action as well as phytosanitary measures were immediately conduced (EPPO, 2002b; EPPO, 2003), it was also recorded in Austria (EPPO, 2002c), Italy (EPPO, 2009a, 2010), Spain (EPPO, 2006), the Czech Republic (EPPO, 2008), Hungary (Petroczy & Palkovics, 2006) and Switzerland (Michel, 2009). Isolated outbreaks were reported in 2009 on peach and nectarine orchards in

Slovenia (Oresek *et al.*, 2010). In Spain (EPPO, 2006), immediately following the identification of *M. fructicola* in a peach orchard, other orchards in the same valley were sampled intensively for potential tree and ground sources of primary *Monilinia* inoculum before and during three growing seasons between 2006 and 2008. Mummies on trees were found to be the main source of primary inoculum (Villarino *et al.*, 2010).

Because chemical treatments have limited effectiveness, physical techniques, such as warm water applications, have been developed to control the development of rotting after harvest (Anonymous, 2009; Karabulut *et al.*, 2010). Simulating models to predict the appearance of brown rot could help to improve disease management (Navrozidis *et al.*, 2008). Michailides *et al.* (2010) underlined the importance of epidemiological studies because reducing the source of inoculum can reduce the incidence of latent infection of fruit with the ultimate result in reducing postharvest disease.

Climatic conditions in the EU are favourable for *M. fructicola* establishment (Van Leeuwen *et al.*, 2001). A weather-based model was tested to improve brown rot management by monitoring infection risk (Holmes *et al.*, 2008). Van Leeuwen *et al.* (2001) published a Pest Risk Assessment on *M. fructicola*. A diagnostic protocol for *M. fructicola* published by EPPO in 2002 was revised in 2009 (EPPO, 2009b); this quarantine pathogen was reported in France (EPPO, 2002a), Austria (EPPO, 2002b), Spain (EPPO, 2006) and the Czech Republic (EPPO, 2008), and is now on the EPPO A2 list.

A number of organisms can interact with *M. fructicola*. Microflora of fruit surfaces have been the best source of antagonists against fungi causing post-harvest decay of fruit. Janisiewicz *et al.* (2010) studied the potential of yeasts and bacteria for biological control of brown rot. Fiori *et al.* (2008) described *Pichia angusta* as an effective biocontrol yeast, while Chan & Tian (2005) studied the mode of action of two other antagonistic yeasts. Zhou & Sholberg (2001) provided an overview of the bacteria (including *Bacillus subtilis*, *Enterobacter aerogenes* and *Pseudomonas syringae*) that have been tested for the management of *M. fructicola*. *In vitro* experiments showed that the fungus *Trichoderma viride* could inhibit *M. fructicola* mycelial growth (El-Sheikh Aly *et al.*, 2000). *Epicoccum nigrum* was also studied as a biocontrol agent against brown rot in stone fruit (De Cal *et al.*, 2009).

Detection and monitoring

To prevent the entry and spread of the brown rot fungus *M. fructicola*, both imported (stone) fruits and nursery stock must be inspected. In European countries where surveys are conducted, visual examinations are performed in nurseries and in stone fruit production orchards to select samples with possible symptoms for immediate testing. Final identification is based on species-specific primers and on methods described in the EPPO diagnostic protocol (EPPO, 2009b). In Germany, infected stone fruits were tested specifically for *M. fructicola* by nested PCR (Albert *et al.*, 2004). In Hungary, symptoms of brown rot were observed on imported peaches and the pathogen was identified as *M. fructicola* on the basis of morphological and molecular characteristics (according to EPPO diagnostic protocol PM 7/18), as well as a pathogenicity test (Petroczy & Palkovics, 2006).

To distinguish the quarantine pathogen *M. fructicola* from other brown rot agents (*Monilinia fructigena* and *Monilinia laxa*), an electrophoresis method using total mycelial protein SDS-PAGE was developed in Italy (Belisario *et al.*, 1999). Hughes *et al.* (1996, 1998) first described monoclonal antibody-based identification techniques before molecular methods were developed and used directly on symptomatic fruits (Hughes *et al.*, 2000; Ioos & Frey, 2000). Cote *et al.* (2004) successfully tested a multiplex PCR to differentiate *Monilinia* species. A synoptic key based on the examination of cultural characters was also produced (Lane, 2002) to differentiate the three *Monilinia* species.

Recently, an automated DNA extraction method combined with a multiplex real-time PCR based on TaqMan chemistry was developed for fast, convenient, reliable and specific detection (van Brouwershaven *et al.*, 2010). Compared with manual DNA isolation followed by a conventional PCR, this method gave improved results, with the detection rate increasing from 65 to 97%.

Pathways and commodities

Imported stone fruits and nursery stock are the main pathways for *M. fructicola* introduction. Van Leeuwen *et al.* (2001) stressed that the fungus can survive transit and easily go unnoticed in the huge volume of fruit imports. The brown rot fungi of fruit crops have a wide host range, comprising fruit and ornamental crops of the family *Rosaceae*. *Monilinia fructicola* is reported to occur widely in stone fruit crops (peach, apricot, etc.) and the import of plants for planting of *Prunus*, *Malus*, *Pyrus*, *Cydonia* and other Rosaceae presents the major pathway for introduction into the EU (Van Leeuwen *et al.*, 2001).

Monilinia fructicola survives as mycelium in mummified fruits, twigs and branches. Apothecia (specific fructifications) may be found in the field and may play an important role in pathogen establishment (Van Leeuwen *et al.*, 2001). The pathogen was also reported on pome fruit (Duchoslavova *et al.*, 2007). In close proximity to *M. fructicola*-infected mummified fruits of cherry and plum trees, isolates from mummified blackberry fruits were identified as *M. fructicola* based on morphological and growth characteristics, as well as two specific PCR tests (Hinrichs-Berger & Muller, 2010).

A survey conducted on *Vitis vinifera* in Canada to determine the incidence of fruit pathogen in wine grapes

detected *M. fructicola* based on morphological characters and DNA sequence data (Sholberg *et al.*, 2003).

The commodities that are most likely to be responsible for international spread of the pathogen are rooted plants and fresh fruits (EPPO, 2009b).

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Perspectives

The new problems created by invasive alien species demand a novel approach to risk management, from prevention to eradication measures (Baker *et al.*, 2005). Regarding surveillance, some techniques that are under study are discussed below, and may contribute to a substantial change in the detection procedures for quarantine organisms.

The main requirements for viable new detection tools or methods are that they are (a) simple to use and sufficiently robust, so that reliable and reproducible results can be obtained by non-specialist staff; (b) generic, suitable for the detection of a range of pathogens/pests; (c) rapid and not causing undue delays in detection; (d) of low specificity when first applied to enable detection of new variants or species; and (e) specific, sensitive, rapid and easy to perform when confirming the initial diagnosis.

Volatile organic compounds

As far as pathogen and pest defence is concerned, it is well known that plants/fruits actively respond to damage with the emission of a bouquet of biologically active volatiles, typically dominated by compounds that are not emitted when they are undamaged or mechanically damaged (Dicke, 1999; Llusià and Peñuelas, 2001; Baldwin *et al.*, 2002). Therefore infested plants might show a peculiar and unique olfactory fingerprint that opens new perspectives for a volatile organic compound (VOC)-based diagnosis of plant diseases. Ethanol, for example, is frequently a major breakdown product of foodstuffs when bacteria or fungi proliferate. When foodstuffs such as grain, potatoes or fruit are stored in bulk, disease can spread rapidly once an infection has become established, and may cause significant losses (de Lacy Costello *et al*., 2000). The instrumental methods for determining odours include gas chromatography/mass spectrometry (GC-MS), electronic nose (e-nose), laser-based spectroscopy and proton transfer reaction mass spectrometry (PTR-MS). Dogs have also been trained to sniff out quarantine pests, and some experience in this already exists for longhorn beetles of the genus *Anoplophora*.

Remote visual signals

The reflectance of vegetation varies across the electromagnetic spectrum. Thriving vegetation is largely green due to the absorption of blue and red radiation by chlorophyll in the leaves during photosynthesis. In contrast, in the near-infrared region, healthy plants are highly reflective because of the scattering that takes place between the spongy mesophyll cells of the plant. Few studies have looked at the spectral responses of diseased plants in the near-/mid-infrared. Remote sensing using Infrared Fourier transform spectrometers offers a method to rapidly identify diseased fruit and vegetables, either at border inspection or from a remote sensing platform.

A range of new technologies for detection are becoming available that may ultimately provide solutions for quarantine screening. X-ray technology has been used for a long time to detect alien species of insects associated with seed trade (Roques, 2001). Often, the pests are hidden. Near-infrared (NIR) spectroscopy can be used to detect infested fruits (although at present accuracy is not sufficient for identification to species level; Toyoshima *et al.*, 2006). Fine-resolution dedicated micro-magnetic resonance imaging (MRI) apparatus using echo methods can also be applied to detect the infestation of small fruits by insect larvae (Koizumi *et al.*, 2010).

Acoustic signals

Insects developing within a substrate produce vibrations when moving, chewing or during other activity. Vibrations are transmitted through the substrate and radiate in the surrounding air as airborne sound. For several decades, different technologies have been used to detect these signals, either for basic research into the physiology, behaviour and ecology of the emitter, or to provide a tool for sensing invisible hidden pests. Detection of movement and feeding activity has been carried out for many insect taxa, particularly for larvae of beetles and moths and for termites. Structure-borne sound has been detected by bimorph elements (linear piezoelectric elements), piezoelectric transducers (planar piezoelectric elements of various sizes, circular and square), microphones and accelerometers. The analysis of temporal and spectral characteristics of recorded signals showed differences among species and among individuals of different size. The number of substrate-boring species where signals can be efficiently extracted from environmental background noise increases with improved technology and software for sound analysis, yielding the opportunity for a wider use of acoustic technology as a diagnostic tool.

Improved pest trapping, especially with automatic devices

Trapping has been used for a long time to assess the presence and density of insect populations, and received even more interest in the framework of integrated pest management programmes, and with the development of this concept related to the economic threshold of damage

(Muirhead-Thompson, 1991; Southwood & Henderson, 2000). In the context of biological invasions, trapping assumes a fundamental importance in the arrival and establishment phases of a pest, as it may provide information not only about the introduction of a new organism, but also in relation to the threshold of establishment. It is known that population dynamics of invasive organisms are strongly influenced by Allee effects and stochastic dynamics, both of which may lead to extinction of low-density populations (Liebhold & Tobin, 2008). As strategies to eradicate newly established populations are often based on suppressing populations below Allee thresholds, availability of reliable trapping devices is essential. Models predicting the probability of entry and establishment are largely dependent on quantitative assessments of population density (Jerde & Lewis, 2007). Methods used to detect quarantine and exotic pests at points of origin and of entry are extremely numerous and vary greatly with the target organism. This review specifies a number of ways in which trapping can be optimized for different target species. However, it still remains a difficult task because of the high cost of deployment. Automatic traps, able to detect the catch of the target species and to send the information via a remote control system to the inspection officer, could be a new frontier. Pilot projects have shown that the process is feasible, but needs improvement (Guarnieri *et al.*, 2011).

Confirmation of pest identity using molecular markers

Identifying invertebrate pests and fungal/bacterial pathogens to species level using morphology alone can be time-consuming and requires specialist skills and knowledge. Many invertebrates can be morphologically cryptic in their juvenile stages, and may require culturing to arrive at a positive identification, a process that can take many weeks. Furthermore, identification cannot be made if these samples are dead on arrival at the laboratory, or die during culturing. DNA barcoding could become a valuable tool in this arena (Boonham *et al.*, 2008). In addition to assigning unknown individuals to species and enhancing the discovery of new species, the technique can be used to identify unknown specimens. Given a validated dataset of sequences obtained from morphologically identified species, an unknown individual or juvenile may be identified by placing its sequence in the tree and seeing which species it clusters with. In contrast to the more traditional molecular diagnostic 'tests' (usually based on PCR), which produce a yes/no answer (where the latter is often an unhelpful result) for the specific assay used, DNA barcoding can be thought of as a molecular identification tool. The future of DNA barcoding as an application in the plant health arena will ultimately be determined by the availability of validated databases of sequences.

The FP7 project QBOL (Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health) is addressing this problem for quarantine organisms in the European Union. It participates in the Consortium for the Barcode of Life (http://www.barcodeoflife.org/), which coordinates many barcoding projects. Of notable interest are the Mosquito Barcode Initiative, the Tephritid Barcode Initiative and the International Network for Barcoding Invasive and Pest Species.

A further application of DNA barcoding data that is currently emerging is the use of short sections of the barcode as probes on microarrays (Hajibabaei *et al.*, 2007). This has the potential for large-scale microarrays containing probes for thousands of species on one slide; the future of this technique, however, lies in the development of inexpensive and validated arrays. The cost of direct sequencing compared with even modestly sized arrays is weighted heavily in favour of the sequencing approach.

Molecular technologies for detection and monitoring are already becoming available, for example DNA barcoding and, soon, environmental barcoding (Hajibabaei, 2009). Customs authorities are important potential end-users for species determination by DNA barcoding. Routine DNA barcoding combined with automated trapping methods for Lepidoptera will greatly enhance detection and monitoring efficiency and accuracy in the near future. As impied above, a comprehensive database of COI sequences is required in order to identify species reliably (DeWaard *et al*., 2010), and the main limitation is the time required to obtain the sequences.

One of the major challenges to be resolved is that of sensitivity (Boonham *et al.*, 2007). Currently, most molecular techniques either amplify all the nucleic acid from the sample (and do not increase the proportion of the target organism in the mixture), or involve the use of biased amplification techniques such as PCR, with the associated problems of multiplexing. Methods are needed that can give specific amplification of the target organism (signal) against the background of the host material (noise).

Sentinel plants

A major issue in pest risk assessment is that the most serious invasive species are often not pests in their region of origin, partly because their original host plants are more resistant to the species than the newly encountered hosts (Britton *et al.*, 2010). A novel method to detect new potential pests in their region of origin, before they are introduced into a new continent, is based on the use of sentinel plants. This method consists of two actions: (a) planting sentinel European plants in other continents and surveying damage caused by indigenous organisms; and (b) surveying damage to European plants already planted in arboreta in these continents. These two methods are complementary, since the sentinel tree methods provide statistically robust data on pests colonizing young trees, but are logistically difficult to implement. In contrast, arboretum surveys are easier to carry out and can provide data on pests attacking mature trees. However, observations in arboreta are not

easy to analyse statistically because they often refer to observations on single trees that may not be representative of the tree species variability. Both methods require strong local links with entomologists and pathologists to be successfully implemented. The two approaches have resulted in a first list of potential pests from China and Siberia related to woody plants (Kenis *et al.*, 2011). Some are already identified and can be the target of new PRAs, while others need further research on their identification and impact.

Another use of sentinel plants is associated with the deployment of susceptible plants inside or around delimited outbreaks, to assess the persistence or the spread of the organism, as done with citrus canker in Florida (Parnell *et al.*, 2009) and Asian longhorn beetle in Italy (Herard *et al.*, 2009).

Statistics and epidemiology

There is growing interest in how much effort should be invested in detection of invasive alien species, and how the traditional methods of eradication (e.g. culling) can be combined with detection. One recent example is provided for the sudden oak death in California (Ndeffo Mbah & Gilligan, 2010). Using a combination of an epidemiological model for two host species with a common pathogen together with optimal control theory, these authors address the problem of how to balance the allocation of resources for detection and epidemic control in order to preserve both host species in the ecosystem. Contrary to simple expectations, the results show that an intermediate level of detection is optimal. In addition, a slight change in the balance between the resources allocated to detection and those allocated to control may lead to drastic inefficiencies in control strategies. The pattern can be changed by a shift in efficiency of the detection methods due to adoption of new technology.

Diagnostic networks

Development of coordinated, robust diagnostic networks that share expertise and technical capacity toward a common goal offers a solution to resource limitations and an opportunity to improve the quality and quantity of these services. Standardization and communication of laboratory practices and protocols is increasingly important, as international commerce requires mechanisms to define and ensure safe and pathogen-free trade. Valid and internationally supported diagnostic methods must be employed to encourage trust in test results. In 1995, van Halteren (1995) called for development of a diagnostic network that would serve national plant protection services in Europe. The network, comprised of interdisciplinary working groups, would develop standardized diagnostic procedures, share expertise, and expedite the adoption or adaptation of new diagnostic techniques. Since 1998, such an attempt has been made with the establishement by EPPO of Panels on diagnostics (Petter *et al.*, 2005). More than 100 diagnostic protocols have been adopted at EPPO level for the diagnosis of regulated plant pests and pathogens. In 2006, a database on diagnostic laboratories and their related expertise was established and is now accessible online (Roy *et al.*, 2010). The use of the diagnostic protocols adopted by EPPO has also been monitored, indicating a positive impact (Petter & Suffert, 2010).

Motivation for development and implementation of a laboratory quality assurance system may be client-driven, or as a result of legislative action or other rule-making authority. Development of standard operating procedures is one step in a process to ensure reliable diagnosis; accreditation of laboratories is a process of assuring quality management within the laboratories. A laboratory must be able to document that procedures are applied in appropriate facilities and infrastructure, using appropriate and properly calibrated instrumentation, and by trained personnel. An EPPO Standard that includes specific quality management requirements for plant pest diagnostic laboratories preparing for accreditation has been adopted (EPPO, 2007). Plant Health Australia (Moran & Muirhead, 2002) noted that its strategic plan to establish a diagnostic laboratory network must include a quality assurance (QA) framework. Several models for QA exist: one being adopted internationally is ISO 17025 accreditation. Documentation, proficiency demonstration, and calibration and maintenance of instrumentation to the level required by ISO 17025 are time-consuming and require resources to dedicate personnel to accomplishing the documentation. Most plant diagnostic laboratories lack sufficient funding to accomplish the full accreditation to ISO 17025, but components of the system are applicable to even the most basic laboratories. The application of a flexible scope may be more appropriate than strict adherence to full ISO 17025 for laboratories that must respond to new and/or changing samples or procedures, such as those in plant diagnostic laboratories (Camloh *et al.*, 2008). Recently, QA and accreditation was discussed at a meeting of diagnostic laboratories in EPPO (http://archives.eppo.org/MEETINGS/2011_conferences/heads_labs.htm), and the establishment of a discussion platform has been suggested and is under preparation by the EPPO Secretariat. Further development is expected with the EU coordinated action EUPHRESCO (http://www.euphresco.org/).

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Une revue des techniques de surveillance des organismes nuisibles pour détecter les organismes de quarantaine en Europe

Cet article passe en revue les méthodes les plus utilisées pour détecter les organismes nuisibles aux plantes appartenant à des groupes d'organismes envahissants qui ont une grande importance économique, comme les

Coleoptera (scolytes, buprestides foreurs du bois, chrysomèles, longicornes, charançons), Diptera (mouches des cônes, mouches des fruits), Homoptera (pucerons, cicadelles et psylles, aleurodes), Lepidoptera (papillons), Thysanoptera (thrips), bactéries (pourriture brune de la pomme de terre *Ralstonia solanacearum*), champignons (chancre du pin *Gibberella circinata*, moniliose *Monilinia fructicola*). Les perspectives pour les méthodes de détection sont discutées, avec une référence particulière à l'importante augmentation dans les volumes, les types de marchandises et les origines de échanges commerciaux de matériel végétal à partir de pays tiers, l'introduction de nouvelles cultures, l'expansion continue de l'UE avec l'addition de nouveaux pays à ses frontières et l'impact du changement climatique affectant les frontières géographiques des organismes nuisibles et de leurs vecteurs.

Обзор методов обследований для выявления карантинных вредных организмов в Европейском союзе

В этой статье рассматриваются наиболее часто используемые методы, позволяющие выявлять вредные для растений организмы, принадлежащие к различным группам инвазивных организмов, имеющие высокую экономическую значимость, такие как жесткокрылые (жуки-короеды, златки, листоеды, усачи, долгоносики), двукрылые (шишковые семенные и плодовые мухи), равнокрылые (тли, цикадки, листоблошки и белокрылки), чешуекрылые (бабочки), пузыреногие (трипсы), бактерии (картофельная бурая гниль *Ralstonia solanacearum*), грибы (рак сосны *Gibberella circinata*, бурая гниль *Monilinia fructicola*). Рассматриваются перспективы развития методов выявления, при этом внимание заостряется на значительном увеличении объемов торговли, на разнообразии типов товаров и источников отправки растительных материалов, происходящих из третьих стран, на завозе новых культур, на продолжающемся расширении ЕС с добавлением новых стран к его границам и на воздействии климатических изменений, затрагивающих географические границы распространения вредных организмов и их переносчиков.

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Glossary

BIO-PCR: enrichment of targeted bacteria by incubating samples on general or selective media followed by polymerase chain reactions with specific primers for detection of plant pathogenic bacteria
DAS-ELISA: double antibody sandwich-enzyme linked immunosorbent assay
GFP-tagging: tagging using a green fluorescent protein-expressing plasmid
IF: immunofluorescence
IFAS: indirect immunofluorescence antibody staining
IGS PCR: DNA-based method using markers such as the ribosomal intergenic spacer (IGS) region
IIF: indirect immunofluorescence
LAMP: loop mediated amplification
MDA-PCR: multiple displacement amplification-polymerase chain reaction amplification
Multiplex PCR: multiple primer sets within a single polymerase chain reaction mixture to produce amplicons of varying size that are specific to different DNA sequences
Nested PCR: two sets of primers, used in two successive runs of PCR, the second set intended to amplify a secondary target within the first run product
PCR: polymerase chain reaction
RFLP: restriction fragment length polymorphism
RT-PCR: real-time PCR, a quantitative nucleic acid amplification method (improvement of conventional PCR)
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis, a technique widely used in biochemistry, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight)
SYBR-green real-time PCR: method using SYBR-Green I (Applied Biosystems) as a fluorescent dye, which intercalates specifically with double-stranded DNA during the extension phase of the PCR
TaqMan method: commercial name for a fluorescent probe detection method.