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Abstract:

An alternative to the conventional delivery methods of pesticides is needed to limit risks for consumers, users and the environment. Managing pests and diseases in orchards, forests and urban environment using trunk injection of plant protection products is a promising strategy to reduce the risks associated with spraying. This environmentally friendly method was developed in the years following the emergence of phytosanitary problems and new scientific knowledge in the field. Recently, renewed interest in the trunk injection method has emerged following the apparition of new biological control agents and technologies which are more tree-friendly. Here we compare existing injection devices and their impact on trunk injection. We focus on the advantages and drawbacks of endotherapy with respect to environmental concerns and the risks for tree and human health. We also discuss the factors that influence the effectiveness of the

trunk injection including the characteristics of the agrochemicals and biological control agents, tree anatomy and physiology. The match between pest or disease occurrence and the timing of the injections also has an influence on the success of this alternative treatment method.

Keywords: Tree trunk injection, Pest management, Disease control, Endotherapy, Plant protection products

1. Introduction

In orchards and forests, applying pesticides using conventional methods - spraying or soil drenching for example - is the currently most common approach used for pest management. Although pesticides are useful to treat pests, they can have several collateral effects, all the more when they are misused (Perry et al., 1991). These include pollution of environment, risks for users and consumer exposure. Foliar spraying is the most common way of applying pesticides to trees but the efficiency of spraying is limited by losses due to drift, and spraying is difficult or impractical for large trees, such as ash or chestnut trees, and is sometimes restricted or prohibited in the proximity of urban area (Aćimović et al., 2016; Wise et al., 2014). Legislation in the USA and Europe has led to the elimination or restriction of the use for many pesticides with the aim of making pesticide use consistent with the concept of sustainable development, meaning alternative approaches are needed (Aćimović et al., 2014). Among these, tree trunk injection is a promising way to deliver agrochemicals in many tree species while reducing environmental impacts and eliminating spray drift (Wise et al., 2014). This application method can be used in forests, orchards and urban area such as gardens and parks (Coslor et al., 2018a; Doccola et al., 2012; Ferracini and Alma, 2018; Kobza et al., 2011). Endotherapy enables plant protection products to be supplied directly to the vascular system to avoid root or cuticle barriers and to disperse the plant protection products inside the plant

(Fettig, 2013a). This method is used to deliver most plant protection products, provided the characteristics are compatible with apoplatic transport to obtain a good uptake and minimize phytotoxic effect (Bromilow and Chamberlain, 1988). It can deliver agrochemicals and biological control agents, and can thus be classified as an environmental friendly way of controlling bacteria, fungi, nematodes, insects, and phytoplasma (Aćimović et al., 2015; Byrne et al., 2012; Hu and Wang, 2016; Percival and Boyle, 2005). Trunk injection can also allow to deliver growth regulators, defense activators, plant bio-stimulant and fertilizers (Aćimović et al., 2015; Bahadou et al., 2017; Dal maso et al., 2017; Fernandez-Escobar e al., 1993). After outlining the history of trunk injection and the recent advances, the different devices used to inject trees are reviewed. The factors that influence the effectiveness of the method along with its advantages and potential drawbacks are discussed. Finally, this review article address the future research needs in the field.

2. History and recent advances

Injecting chemicals into the trunks of trees has a long history with disparate results. Several attempts have been made to use the technique over the centuries but without success. In the 15th century, Leonardo da Vinci was the first to attempt to inject trunk. He introduced arsenical and other poisonous solutions in apple trees, through bore holes, in order to make fruit poisonous (Roach, 1939; Stoddart and Dimond, 1949). More recently, in the 19th century, new experimentations in the field of plant injection brought developments of the method. Hartig, in 1853, was the first to inject liquid into a hole from a container outside the tree. Sachs (1894) injected iron salts in solution with this method to correct a deficiency disease (Roach, 1939; Stoddart and Dimond, 1949). In 1894, Ivan Shevyrez was the pioneer in the way to use tree injection for purposes of pest control in the USA, followed more recently by American, French, Italian, English and German workers (Rumbold, 1920). However, in this century lack of knowledge in basic science was an obstacle to understanding experiments in trunk injection. Roach and Rumbold compiled works between the 12th century and the early 20th century (Roach, 1939; Rumbold, 1920). The most widely used substances in that period were dyes and salts. More recent research in the 20th century produced new knowledge in botany, plant physiology, agriculture and forestry. How tissues healed after injury was better understood and described as compartmentalization by Shigo (Shigo, 1977). The 20th century also saw the emergence of the cohesion-tension theory for the movement of water in trees by Dixon-Joly and Askenasy (Dixon and Joly, 1895). Renewed interest in trunk injection emerged following the spread of dutch elm disease (*Ophiostoma Ulmi* Biusman) in the USA in the 1940s (Burkhard et al., 2015; Perry et al., 1991). Management of dutch elm disease fungus by injection of fungicides have shown good results (Haugen and Stennes, 1999; Karnosky, 1979; Perry et al., 1991). To identify the path of water conduction in trees, Kozlowski injected dyes into the stem of forest trees (Kozlowski et al., 1967). In the 1990s and 2000s, the spread of invasive and new pests and diseases across the world revived research on trunk injection. In the USA, the method is mostly used to treat tree-killing insects such as the emerald ash borer (*Agrilus planipennis* Farimaire) (Grimalt et al., 2011), longhorn beetle (*Anoplophora glabripennis* Motschulsky) (Ugine et al., 2013) and hemlock woolly adelgid (*Aldelges tsugae* Annand) (Doccola and Wild, 2012). Endotherapy has also been used to control the horse-chestnut leaf miner (*Cameraria ohridella* Deschka et Dimic) in Europe (Kobza et al., 2011), pine wilt nematodes (*Bursaphelenchus xylophilus* Steiner et Buhrer) in Asia and Europe (Sousa et al., 2013) and the red palm weevil (*Rhynchophorus ferrugineus* Olivier) in the Middle East, North Africa and Europe (Burkhard et al., 2015). Furthermore, successful control of fungicides by trunk injection has already been reported (Amiri et al., 2008; Dal Maso et al., 2015, Percival and Boyle, 2005). Trunk injection of phosphite against *Phytophtora* species has become a common practice in forests and orchards (Akinsanmi and Drenth, 2013; Garbelotto et al., 2007).

More recently, there has been renewed interest in trunk injection as an alternative to spraying in orchards and in landscapes where other methods cannot be applied or are ineffective, and to limit non-target exposure (Aćimović et al., 2015). For instance, trunk injection has been studied to control fire blight (*Erwinia amylovora* Burrill) in apple trees and downy mildew (*Plasmopora viticola* Berk. et Curt.) in vines (Aćimović et al., 2014b; Düker and Kubiak, 2009). Trunk injection of antibiotics and plant activators (i.e. SAR inducers) appears to be the only effective method available to control citrus huanglongbing caused by the systemic pathogen *Candidatus liberibacter* Garnier (Hu and Wang, 2016, Hu et al., 2017; Puttamuk et al, 2014; Shin et al., 2016). Systemic acquired resistance (SAR) can be activated by either the pathogen infection itself or by applying chemical inducers to the plant. SAR inducers are usually applied as foliar sprays or soil drenching (Wise, 2016), but some authors have investigated the delivery of SAR inducers to the vascular system by trunk injection. Aćimović et al. (2015) reported significant control of fire blight in apple trees by injecting acibenzolar-S-methyl and potassium phosphite. Similarly, Hu et al. (2017) tested several SAR inducers including salicylic acid, oxalic acid, acibenzolar-S-methyl and potassium phosphate, applied by trunk injection to control citrus huanglongbing. Results showed positive control of the disease. As acibenzolar-S-methyl and salicylic acid are sensitive to environmental conditions and to photodegradation, application by trunk injection may avoid these problems (Hu et al, 2017).

The injection of new plant protection products compounds, RNAi and bacteria, has emerged in recent years and is expanding. Trunk injection of RNA molecules is an innovative method of control by targeting insect pests with lethal genes (Dalakouras et al., 2018; Hunter et al., 2012; Joga et al., 2016). Endophytic bacteria and fungi are promising biological control agents for trunk injection. They have been shown to produce good results against *Phytophtora* species on *Quercus robur* L. and *Fagus sylvatica* L., and for fire blight control in pear and apple (Bahadou et al., 2017; Berger et al., 2015).

3. Injection as an alternative to spraying?

Foliar spraying is the most frequently used way of applying pesticides for pest management in trees. However, the limits of spraying are extensive pesticide losses. According to Pimentel (1995), only 0.4% of active substance actually reaches the target pest. However, the operational target is the canopy and losses depend on the type of vegetation, the growing season, the weather and the sprayers used. Atmospheric drift consists of droplet dispersion during spraying, and of pesticide vapors during and after spraying (Gil and Sinfort, 2005; Lichiheb et al., 2016; Van den Berg et al., 1999; Zivan et al, 2016). Drift is greatly affected by wind conditions (Cross et al, 2001a). Droplets and drips from the tree contribute to ground drift (Deskeyser et al. 2014; Grella et al., 2017). For example, widely used equipment like axial fan sprayers result in large quantities of product deposited on the ground (Cross et al. 2001a). In vineyards, Bonicelli et al. (2010) showed 30% to 40% of air dispersion, whatever the stage of development of the vines. In the case of a high density canopy, ground drift was reduced by from 40% in the early stages to 10% in July when the canopy was most dense. In orchards, several authors reported losses of more than 50% of the spray due to the use of axial fan sprayers (Cross, 1991; Herrington et al., 1981).

Such environmental contamination and inefficient use of pesticides is no longer acceptable. Much effort has been invested in modifying existing axial fans and in adapting sprayers to structure of canopies (Duga et al. 2015; Khot et al. 2012). New practices and new spraying equipment can reduce losses by up to 67%, although losses remain significant (70% in some cases) (Holownicki et al., 2000; Pergher et al., 2018). New sprayers including tunnel and recycling sprayers can reduce droplet drift by collecting losses from the canopy (Pergher et al., 2013, 2018). The use of low-volume sprayers reduces losses but increase the variability of leaf deposit because it more specifically targets the plucking surface (Cross et al., 2001b). The volume of water does not affect total deposits, only percentage surface coverage (Wise et al., 2010). All these problems are amplified in the case of tall trees (10 m and above) such as chestnut, pecan or urban trees that are sprayed using ground-based air-blast sprayers. Spray deposits are considered to decline with tree height (Bock et al., 2013; Bock et al., 2015). In one of the rare studies on tall trees, Bock et al. (2015) showed that the percentage of deposit depends on the height of pecan leaves in the canopy: spray coverage ranged from 73.5% at 5 m to 0.02% at 15 m, even if no linear relationship was identified between the height of leaf sampling and percentage coverage.

Soil drenching is considered as an alternative to spraying and can reduce chemical losses. This method involves applying chemicals to the soil around the tree for root uptake (Hu et al, 2018). Soil drenching is used for chemicals like neonicotinoid insecticides to treat Florida citrus to control the Asian citrus psyllid (*Diaphorina citri* Kuwayama) and citrus leafminers (*Phyllocnistis citrella* Stainton), which are linked to the spread of citrus huanglongbing and citrus canker diseases, respectively (Fletcher et al., 2018; Rogers, 2012). Soil application is also used for the systemic acquired resistance inducer, acibenzolar-S-methyl (Graham and Myers, 2016). However, the fraction of chemicals, for example, of imidacloprid, uptaken by plants can be low and the rest remain in the soil for a long time (Fletcher et al., 2018; Laurent and Rathahao, 2003). The remaining fraction can have adverse effects on soil arthropods, as it is prescribed for the control of soil parasites (Altmann, 1990). Soil drenching is also limited by the need to apply high rates of chemicals and by soil microbial degradation of the active substances (McCoy, 1976; Hu and Wang, 2016).

Due to these risks for the environment and for human health, alternative methods to spraying and soil drench, such as trunk injection, are needed, and current European legislation limits or prohibits pesticide spraying in the proximity of urban areas (Directive 2009/128 CE), reinforcing the interest in alternatives to spraying.

Tree trunk injection has many advantages making this integrative pest management method an interesting alternative to spraying and soil drench. At equivalent dosage, trunk injection provides a higher quantity of plant protection products to trees because the whole dose is delivered in the sap flux. That avoids soil deposition, drift losses or photolysis and microbial degradation at leaf surface (Doccola and Wild, 2012; Fidgen et al., 2013). The closed system reduces non-target impacts and user exposure (Fettig et al., 2013b). Injection also controls borers that feed under the bark where compounds sprayed onto the surface of trees cannot penetrate in sufficient concentrations (Doccola and Wild, 2012).

One advantage of trunk injection may be the persistence of action reported in some studies, meaning one treatment per year – or at even longer intervals - may suffice (Doccola and Wild, 2012; Fidgen et al., 2013). For example, Grosman et al. (2010) evaluated injections of experimental formulations of emamectin benzoate for preventing ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) mortality from colonization by western pine beetle (*Dendroctonus brevicomis* LeConte) in California, and reported three years of protection with a single injection. The cost per tree injection is higher than one spray application because of the labor required, but fewer applications and less solution are required, making it an affordable investment in many cases (Littardi et al., 2013; Wise, 2016).

4. Injection methods and devices

Trunk injection is still evolving but is now tending to be more widely used thanks to technological progress on the devices and formulations adapted for injection. Generally, two parts must be distinguished in injection methods and associated devices: tools to set up the injection port (drilling with bite or needle perforation) and material to deliver the product (open tank, pressurized capsule, syringe, etc.).

4.1. Drill-based versus drill-free devices

There are two categories of injection processes based on the way the hole is made: methods that involve drilling a hole in the trunk with a bit before using the injection device, and needle-based techniques without preliminary drilling. The majority of techniques are drill-based. Drilling can cause friction to the tissues because of the drill speed, causing damage and hence reducing uptake (Montecchio, 2013). Only two needle-based systems have been developed to prevent the potential tree injury: BITE® technology (University of Padova, 2011) and Wedgle® Direct Inject (ArborSystems LLC, NE USA) (Montecchio, 2013; Smith and Lewis, 2005) (Figures 1A and B). BITE is time consuming because it is a passive system. The method relies on intensive sap flow to allow a rapid uptake of the solution. Aćimović et al. (2016) compared injection ports made by drilling and needle-based tree injection technologies on apple trees. The injection port that healed the fastest was shown to be the lenticular port created by BITE®. The slowest was the 9.5 mm drill port sealed with Arborplug® (ArborJet Inc., MA USA). It is necessary to continue to develop non-drilling methods to limit injection wound.

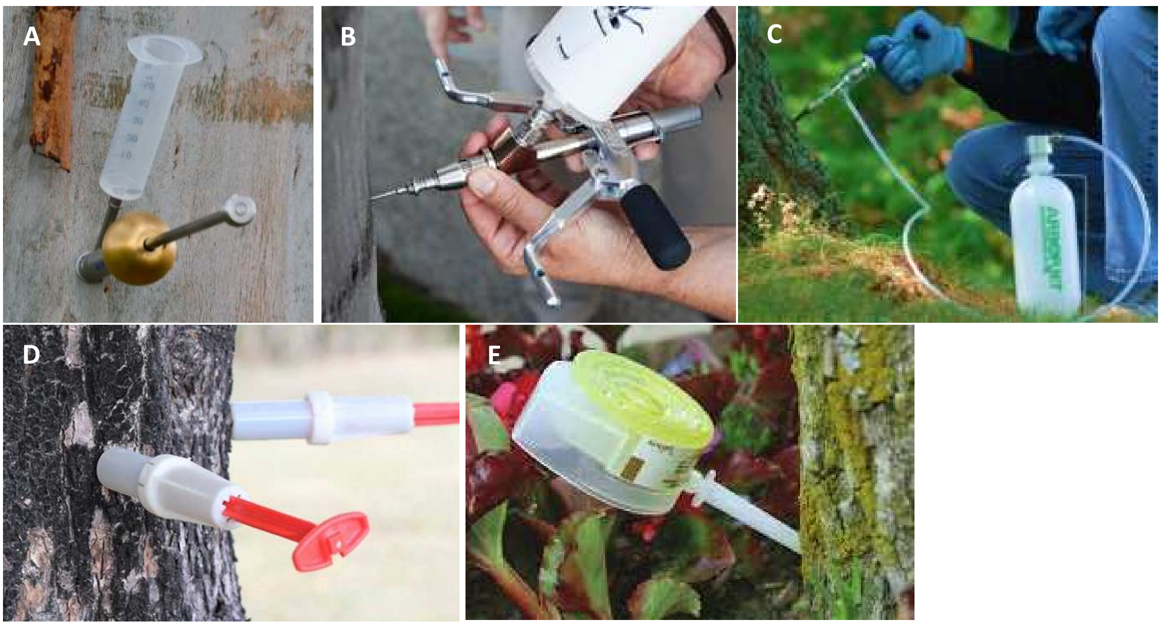

Figure 1: Existing trunk-injection devices. (A) Bite®, (B) Wedgle® Direct-Inject System, (C) Quick-jet® microinjection system, (D) ChemJet® Tree Injector, (E) Mauget®

4.2. Types of delivery tools

After the port is made, the plant protection product in solution can be introduced in the trunk in different ways. Table A and Figure 1 show various devices used for trunk-injection. Most involve a capsule with a canula or a syringe that is inserted into the hole to inject the solution. Other devices are tubing with a pump or a drill coupled with an injector. Acecaps® (Creative Sales, Inc, NE USA) are implants that are inserted into holes drilled into the tree. ArborJet devices (ArborJet Inc, MA USA) use a syringe for single or multiple injections from a central unit with delivery tubes connected to the tree (Figure 1C).

Injection methods also differ in the diameter of the hole ranging from 2 mm to 9.5 mm. The needles and capsule tubes are usually round whereas BITE® (University of Padova, 2011) is a manual, drill-free instrument with a small, perforated lenticular (lentil shaped) blade that enters the trunk. The depth of the injection varies between and within methods since different needle sizes are used that are adapted to the morphology of the target trees. The injected solution moves inside the trunk by natural uptake or is forced under pressure. Most used are high pressure devices whose pressure ranges from 207 kPa to 450 kPa. Viper® (ArborJet Inc, MA USA) and Stemjet® (Chemicolour Industries Ltd., Auckland, NZ) technologies can inject solutions at very high pressure, up to 3000 kPa. With capsules, implants and with the Wedgle® Direct Inject (ArborSystems LLC, NE USA), the volume injected is very small, from 1 to 5 mL, but aside from these methods, the injected volume is generally larger and depends on the specific experiment, not on the device.

Table A: Various methods and devices used in endotherapic experiments.

Kind of hole	Type of technology	Name of the device	Diameter of the hole (mm)	Depth of the hole (mm)	Pressure (kPa)	References
Drilled	Syringe plus drip	Quick-jet® micro-injection system (ArborJet Inc, MA USA)	9-9.5	25.4-120	Hand pressure*	(Aćimović et al., 2015; Byrne et al., 2014; Doccola et al., 2012)
		Tree IV® Micro-infusion System (ArborJet Inc., MA USA)	9-9.5	16-120	207-414	(Aćimović et al., 2015; Doccola et al., 2012)
		Viper® micro-injection system (ArborJet Inc, MA USA)	7.4-9.5	15-40	241-4136	(Aćimović et al., 2015; Doccola et al., 2007)
	Drip	Stemject® (Chemicolour Industries Ltd., Auckland, NZ)	6-8	25-100	3000-4000	(Darrieutort and Lecomte, 2007; Dula et al., 2007)
	Drill combined with injector	Sidewinter® precision injector (Sidewinter Pty Ltd., Australia)	6	40	<4000	(Xu et al., 2009)
	Syringe	ChemJet® Tree Injector (Chemjet Trading Pty. Ltd., Australia)	4.2	25-50	Coil spring*	(Düker and Kubiak, 2009; Shin et al., 2016)
		Avo-ject® syringe injector (Aongatete coolstores Ltd., NZ)	7.5	30	Coil spring*	(Puttamuk et al., 2014)
	Capsule	EcoJect® system (BioForest Technologies Inc., Canada)	5.6-5.8	13-19	379kPa-448	(Booth and Johnson, 2009; Grimalt et al., 2011)
		Tree tech® microinjection system (Tree tech microinjection system FL USA)	3-5	5	65	(Kobza et al., 2011; Percival and Boyle, 2005)
		Mauget® (Mauget Company, CA USA)	4	6-30	Hand pressure*	(Cowles et al., 2006; Raupp et al., 2008; Young, 2002)
	Implant	Acecap® (Creative Sales, Inc, NE USA)	9-9.5	3.2	Natural uptake	(Doccola et al., 2011; Raupp et al., 2008)
Drill free (needle)	Open drip	BITE® (University of Padova, 2011)	3.5	20	Natural uptake	(Dal Maso et al., 2014; Montecchio, 2013)
	Syringe	Wedgle® Direct-Inject System (ArborSystems LLC, NE USA)	2-2.8	4-19	Hand pressure*	(Cowles et al., 2006 James et al., 2006; Rosenberg et al., 2012)

*Pressure of injection is not indicated.

5. Factors influencing the effectiveness of trunk injection

Translocation of agrochemicals inside the tree and the effectiveness of injection are affected by many factors including the properties of the plant protection products, tree anatomy and physiology, the type of pest, environmental conditions and the method used.

5.1. Factors related to application methods

Both the application techniques and the devices influence uptake, i.e. injection pressure, drilling, the location, depth, angle and diameter of the hole, and the shape of the needle or syringe (Hu and Wang, 2016; Sánchez-Zamora and Fernández-Escobar, 2004, 2000).

Currently, an effort is underway to develop techniques that make a clean cut with a smaller diameter and a shallower hole. The smaller the diameter of the hole, the faster the wound heals (Perry et al., 1991). The use of drilling methods has negative side effects such as loss of functionality of adjacent woody tissues and delayed hole closure. Injection methods without drilling limit these effects (Montecchio, 2013). The shape of the needle influences the wound created and the seal mechanism during trunk injection. They can be round shaped, with a screw thread, or lenticular shaped. Lenticular shaped ports, such as BITE® (University of Padova, 2011), may cause minimal injury to woody tissues because they separate the fibers instead of round shape needles that cut the fibers (Montecchio, 2013). Depending on the shape of the needle, cracks may appear, resulting in weak sealing performance (Shang et al., 2011).

Different species have naturally different uptake speeds, consequently it may be useful to use pressure for the slowest species (Navarro et al., 1992). The injection time is influenced by the use of pressure and the volume of compound injected. High pressure and small volumes reduce the time it takes to deliver agrochemicals by trunk injection. High pressure makes it possible to injecting larger amounts of product into the vascular system but this can cause injuries such as cambial damage, bark lesion, that is, bark can separate and split (Montecchio, 2013). Also, if air enter the injection hole causing cavitation, water column is interrupted and the uptake is stopped (Perry et al., 1991). By applying high pressure, leaks of the injected product can appear. The use of seal or septum can limit this problem (Aćimović et al., 2016). By using natural uptake, less injury may be caused but the time required for application is longer and highly dependent on weather conditions (Aćimović et al., 2016; Montecchio, 2013).

5.2. Factors related to the plant protection products

5.2.1. Agrochemicals

Translocation of organic compounds inside a plant depends on the water solubility, lipophilicity, molecular weight, polarity, pH and formulation of the product (Percival and Boyle, 2005). In conventional spraying of pesticides, the most important factor governing the movement of chemicals inside plants is lipophilicity, namely the octanol/water partition coefficient (K_{ow}), due to the need for the molecule to cross the lipid membrane to reach the vascular xylem (Bromilow and Chamberlain, 1988). If the injection supplies the chemicals directly to the xylem sap, this factor could be less important. Cellulose and hemicellulose, the main constituents of vessel walls, are polar and have low absorption capacity of aromatic compounds, such as pesticides (MacKay and Gschwend, 2000). However, lignin, which impregnates polysaccharide polymers of vessel walls, is hydrophobic, sorbs hydrophobic organic compounds and can retain active substances in the vessel walls. Softwoods are composed of 40-44% of cellulose and 25-31% of lignin. Hardwoods have a lower lignin content (18-25%), and are therefore likely to have a lower sorption capacity for lipophilics (MacKay and Gschwend, 2000).

The other main property of a molecule that influences the transfer through bio-membranes is the dissociation constant (pKa) (Sur and Stork, 2003). In phloem, the pH is basic, around 8, while in xylem, the pH is more acidic, about 5. These differences in pH do not affect the distribution of neutral compounds between xylem and phloem, but strongly influence the distribution of ionized compounds (Bromilow and Chamberlain, 1988). Chemicals that are weak acids accumulate in the plant compartments with a high pH, where they are trapped in the phloem (Bromilow and Chamberlain, 1988; Sur and Stork, 2003). Most non-ionized compounds can move freely between xylem and phloem but tend to be carried away by the xylem flux that has the greater sap flow (Bromilow and Chamberlain, 1988). pH values differ among tree species and therefore the partition of active substances between symplastic (phloem) and apoplastic (xylem) vessels depends on species. pH can also vary with the season (Alves et al., 2004).

Other properties of agrochemicals such as molecular weight and partitioning of compounds onto organic matter can also influence systemic transfer (Aitchison et al., 2000; Bromilow and Chamberlain, 1988). Compounds with high K_{oc} values limit long-distance transport in the tree but this process is a function of lipophilicity (Bromilow and Chamberlain, 1988).

Currently, commercial formulations designed for spraying are not necessarily compatible with optimized vascular transfer because the sprayed molecules can remain inside or on the surface of the leaves where the pest is present and do not need to be transported. To modify the physical-chemical properties of active substances and to improve their distribution inside the tree, formulation is essential. To increase the efficiency of the injection, formulations need to deal with water solubility and low K_{ow} (Doccola et al., 2007; Doccola and Wild, 2012; Montecchio, 2013; Young, 2002). Indeed, highly lipophilic compounds (log K_{ow}>4) sorb onto plant solids, including xylem tissues. Compounds with a log K_{ow}=1.8 have an optimal translocation potential (Aitchison et al., 2000; Trapp et al., 1994). Formulation can allow better translocation of compounds with a high K_{ow} and prevent them from bonding to lignin. Very water-soluble chemicals are transported to the leaves but are not available for very long.

5.2.2. Biological pest control agents

Endophytic bacteria can be transported by the xylem and reach the leaves. It seems they use their flagella and/or the transpiration stream to attain the vegetative plants parts. Their size does not interfere with their ability to pass through the vessels elements (Compant et al., 2010). Similarly, the size of the plate and pit holes allows the passage of fungal conidia but there is a lack of knowledge on the characteristics related to the capacity of fungus and bacteria injected to move inside the vessels and reach the target. Efficacy will depend on the mode of protection generated by the bacteria and fungus, efficacy of the agent itself or of its secondary metabolites, hence the need to better understand the mode of action of endophytes, or others bacteria and fungus, to optimize their use (Berger et al., 2015).

5.2.3. RNAi

RNA interference (RNAi) occurs in most eukaryotes that function as regulators of gene expression by targeting specific RNAm sequences. Gene silencing by double stranded RNA (dsRNA) has been used in crop protection. Phloem is considered as the preferential channel for the transport of RNA where it can remain stable over time because phloem sap is free of RNAse (Joga et al., 2016; Melnyk et al., 2011). The size of the plasmodesmata can limit the transfer of RNAi within the plant (Melnyk et al., 2011). Trunk injection can be used to deliver RNAi-based insecticides to control insect pests in trees (Li et al., 2015; Zotti et al., 2017). By contrast to endogenous RNAi, trunk injection delivers double stranded RNA (dsRNA) to the xylem, dsRNA is then translocated inside the tree via apoplastic transport where plasmodesmata are not present (Dalakouras et al., 2018).

Formulations with viruses, bacteria, chemically modified molecules, polymers or liposomes could increase the ability of dsRNA to reach the target and improve efficacy (Dalakouras et al., 2018; Joga et al., 2016). This method would then be more effective for the control of sap-sucking insects than for chewing insects that mostly feed on leaves (Joga et al., 2016).

5.3. Influence of tree anatomy and physiology on the transfer

Some biological factors related to plant physiology and anatomy including tree species, size, health status, xylem architecture and the phenological stage can affect the distribution of the plant protection product (Sánchez-Zamora and Fernández-Escobar, 2000).

As in all plants, tree water flux from roots to leaves is driven by aspiration due to the leaf transpiration, and flux is maintained continuous by capillarity forces according to tension-cohesion theory (Dixon and Joly, 1895; Hacke et al., 2006; Venturas et al., 2017). Injecting the chemicals directly into xylem tissues enables the translocation of compounds via the transpiration stream (Chaney, 1986).

Most of the vascular system is composed of secondary xylem and phloem tissues produced by the vascular cambium. The xylem is made up of different proportions of vessels, tracheids, fibers and parenchyma cells organized differently depending on the tree species (Chaney, 1986; Pallardy, 2010). The properties of the xylem that facilitate the radial and vertical distribution of active substances are high density of vessels, large vessel diameter, increased intervessel contact, high density of intervessel pits and the porosity of the pits (Orians et al., 2005; Zanne et al., 2006).

Broadleaves (hardwoods) and conifers can be distinguished by the anatomy of their vascular system. A gymnosperm, e.g. a pine, spruce, or fir, are non-porous trees. Their xylem is only composed of one type of cell, tracheids. Tracheids range from 10 to 20 µm in diameter with lateral connections, in the form of pits, between the tracheids (Chaney, 1986; Sperry et al., 2006). Because of the small diameter of these cells, the movement of the injected compound is slowed down; there are more resistance points than in large vessels like in xylem types of hardwoods. Conifers also have resin canals in the xylem that can reduce the uptake of the injected compounds (Sánchez-Zamora and Fernández-Escobar, 2004, 2000). They use seven to 10 rings to transport sap, and consequently also for the translocation of the injected compounds (Chaney, 1986).

The xylem of angiosperms is composed of both vessels and tracheids connected to each other and to vessels by small pits (Chaney, 1986). In angiosperms, there are two kinds of xylem arrangements: ring porous trees such as chestnut, ash and elm; and diffuse porous trees, such as poplar, apple and willow (Pallardy, 2010). Vessels are 10 to 200 μm in diameter and up to 10 m in length. They can be both larger in diameter and longer in ring porous trees than in diffuse porous trees (Chaney, 1986; Hacke et al., 2006; Hacke and Sperry, 2001). Vessels in diffuse porous trees are uniformly dispersed among the tracheids in each annual growth ring whereas, in ring porous trees, wider vessels in the xylem are predominant in the early wood while the vessels are smaller in diameter or absent in latewood (Chaney, 1986). Organic chemicals in broadleaf trees move mainly in the one to three outer annual rings (Chaney, 1986; Kozlowski et al., 1967). The kind of xylem influences hole depth. In ring porous xylem, shallow injections are more reliable because 90% of the hydric activity takes place in the current annual ring. Diffuse porous trees also use the three outer rings but the distribution between the rings is more balanced, only 70% of the sap moves via the outer ring, so injecting into more than one ring is ideal (Chaney, 1986; Kozlowski et al., 1967).

Differences in the transport of nutritional resources through plants among genera and species are highly dependent on the xylem pathway from the roots to the leaves. The ascent of sap can be sectorial, preferentially using paths with the most direct vascular connections (Kozlowski et al., 1967; Orians et al., 2005; Zanne et al., 2006). Trees with a great degree of radial sectoriality move resources mainly in the longitudinal plane and have a low radial diffusion. Lateral movement by radial diffusion occurs in trees with a greater degree of integration such as diffuse porous trees (Aćimović et al., 2014, Hu and Wang, 2016, Larson et al., 1994, Tanis et al., 2012). Some species, including elm and apple, have a spiral grain leading to a sectorial winding ascension of the injected compound, that results in good distribution throughout the canopy (Aćimović et al., 2014a; Chaney, 1986; Orians et al., 2005). Some species, including ash, have a straight grain, meaning the compounds follow a sectorial straight transport in line with the location of the injection (Chaney, 1986; Kozlowski et al., 1967). Sectored sap flow leads to irregular distribution of the injected compounds in the canopy resulting in variable control (Byrne et al., 2012; Orians et al., 2004). Because of this architecture, multiple injection ports spaced radially around the stem are required to achieve uniform distribution in the tree canopy. Aćimović et al. (2014) found a minimum of four injection ports were required in 29-year-old apple trees with trunk diameter of 30 cm at a height of 28.5 cm from the ground. A study on citrus trees by Hu and Wang (2016) recommended the use of two injection ports for five year old trees with a trunk diameter of 9 cm 15 cm above the bud union.

After injection, tree metabolism can modify the efficacy of the injected compounds, limiting the length of effectiveness (Tanis et al., 2012). When injected, active substances may be protected from UV degradation or outside biodegradation. However, chemicals could be degraded by plant metabolism, too. First, it can occur in xylem tissues, which is a rich peroxidase tissue for biosynthesis of the lignin polymers. Some chemicals could be trapped in lignin, as bound residues, by copolymerization with lignin monomers. Secondly, they can be degraded in leaf parenchyma by xenobiotic metabolism pathways (Roberts, 2000).

5.4. Influence of weather conditions on uptake in trees

The time of the year and climatic conditions also influence translocation of the compounds after injection. Consequently, atmospheric conditions, i.e. light, wind, relative humidity, and temperature need to be taken into account. Weather conditions such as high humidity and low sunlight have a negative effect on the process of absorption of agrochemicals inside the plant, whereas rain and wind do not slow down the process (Littardi et al., 2013). The amount of vapor pressure in the atmosphere is a major factor because a decrease in vapor pressure increases the transpiration rate. The ideal conditions for stomata in the tree canopy to be open and for a high transpiration capacity are sunny and windy weather with substantial water supply in the soil (Doccola et al., 2007; Fettig et al., 2013b).

The best uptake usually occurs in spring during the most intensive transpiration periods and in summer with the new green growth in the canopy, but multi-season injections, such as an injection in late summer or early fall, can be used to provide protection for the following year (Fettig et al., 2013b).

5.5. Matching pest occurrence and timing of injection

Several factors related to pests can influence the efficiency of injection: the pest or disease itself, the period of occurrence and infestation pressure, or the nature of impacted tissue. First, the pest must be distinguished from disease management due to mobility or impacted tissue. Piercing-sucking insects have to be distinguished from chewing insects or borers. Piercing-sucking insects feed on the sap directly in vascular bundles while chewing insects and borers eat either the whole leaves, or only the parenchyma, and bark or wood. Concerning disease management, a distinction should be made between ecto- or endo-parasites, and in all cases, between fungi, bacteria or viruses. Efficiency depends on which tissues, parenchyma, phloem or xylem tissues, are impacted. In all cases, a good correlation must be found between plant protection products localization, over time or in the tissues, and the location of the parasite inside the tree.

Systemic pathogens, such as those that cause Dutch elm disease (*Ophiostoma Ulmi* Biusman), probably come into contact with injected compounds earlier and at higher concentrations than in the case of diseases limited to the leaves and fruit, such as apple scab (*Venturia inaequalis* Cooke). Indeed, the injected preparations will be at higher concentrations in the xylem vessels where injections are located, and then presumably diluted by the xylem sap or by foliage biomass (Byrne et al, 2014).

It is important to choose the best timing for the injection to ensure the peak concentration of the compound matches the period with the highest pest pressure (Byrne et al., 2014). In the most complete study on this topic, Byrne et al. (2014) showed that the choice of the appropriate stage of the tree is primordial for efficiency of treatment but that timing also depends on the active substance. Due to its rapid distribution within trees, acephate is appropriate to control sudden outbreaks of thrips whatever the flush period. By contrast, imidacloprid is most effective when injected during the mid-flush period and subsequently reaches optimum levels in the leaves when the thrips actively feed on young leaf tissues (Byrne et al, 2014).

To control pests that attack the developing tissues or attack early in the growing season, injections in the fall or in the early spring can insure translocation before damage occurs (Cook et al., 2013). Similarly, Fettig et al. (2014) showed that emamectin benzoate has to be injected into the lodge pine trunk (*Pinus Contorta* Douglas) one year before the protection is needed against mountain pine beetle (*Dendroctonus ponderosae* Hopkins). While the time of the injection the previous year is not important, is must allow good distribution of the active ingredient in the targeted pine tissues (Fettig et al., 2014). A less appropriate time of injection may require increasing the dose of the active substance (Kobza et al., 2011). As infestation may vary from year to year, the number of treatments and the timing have to be adapted accordingly.

What is more, some treatments do not produce good results when applied as a curative treatment, but are efficient preventive measures, in which case the product has to be injected earlier (Berger et al., 2015).

6. Risks related to trunk injection

6.1. Risks for trees

By creating a hole in bark and in the sapwood, injection involves some risks for tree health. Aćimović et al (2016) compared drill- and needle-based tree-injection techniques to investigate port closure, bark cracking and callus formation in apple trees. He showed that port closure took from one year to more than two years and that the lenticular port left by the blade healed fastest. Working in peach trees, Cooley et al. (1992) found no evidence of significant damage to the tree after two years but wounds were not closed by callus formation. Percival and Boyle recorded total wound closure by measuring callus formation at the end of the first growing season in apple trees and English oak (Percival and Boyle, 2005). Doccola et al. (2011) reported that green ash grew over 80% of the injured vascular system in two years with no signs of negative impacts on tree health.

However, wound closure is only one aspect of many physiological responses of trees. Visual observations of the external wound left by injection showed trunk splitting, bark separation, fluxing of sap, and in the inner tissues, wood staining and decay (Aćimović et al., 2016; Perry et al., 1991; Shigo et al., 1977). If high rates of chemicals are used, long term and permanent injuries may occur, including leaf yellowing or leaf death, or reduced fruit yield (Aćimović et al., 2016). Tree health and longevity may also be affected by the wound created by the injection, as the port is an entry point for pathogens and insects (Ferracini and Alma, 2008; Percival and Boyle, 2005; Perry et al., 1991). After injection, wounds are usually compartmentalized by walls that confine the injured tissues, and repeated injection over time can lead to a majority of occluded or walled vessels, making further injections impossible (Shigo, 1984; Shigo et al., 1977; Smith and Lewis, 2005).

Numerous studies have reported no external symptoms of phytotoxic effects associated with trunk injection treatments (Fettig et al., 2013c; Grosman et al., 2010). However, fluxing of sap and bleeding can occur around the injection openings, which could be misperceived and considered unsightly in urban environment and therefore undesirable (Fettig et al., 2013c; Perry et al., 1991).

6.2. Risks for humans and the environment

When spraying methods are used, the main chemical risk is to the workers who do the spraying and who are exposed to high concentrations of agrochemicals. It is clear that injection limits that risk. However, workers can be exposed when handling the product, for example when preparing the spray, or by leaks during injection, especially when high pressure is used.

On the other hand, the risks for consumers of the presence of the chemicals in food can be assessed in the same way as for conventional treatments. In fruit trees, it is crucial to use the optimal amount of the active substance that produces the necessary efficacy with residue levels in fruits below the maximum residue levels MRLs defined by authorities (Directive 2009/128 CE). When acephate is injected into the avocado trunk to control thrips on young fruits, efficient concentrations are found in the fruits but residue levels are below the MRLs at harvest (Byrne et al. 2012). Similarly, when injected in the trunk before blossom, residues of abamectin, emamectin benzoate or imidacloprid in apples are below the U.S. MRLs at harvest, whereas they are still found in the leaves (Coslor et al., 2018b).

Correct application may also prevent toxicity for pollinators exposed to agrochemicals, when sprayed, by contact from drift but also after spraying when pollinators are feeding on the target plant. Studies have shown that most of the residues end up in the foliage but some have been detected, at low levels, in flowers and fruits (Byrne et al., 2014, 2012; Coslor et al., 2018a; Hu and Wang, 2016; Vanwoerkom et al., 2014; Wise et al., 2014). The timing of the injection can be used to control the levels of pesticide to insure residues are below the maximum permitted level in fruits. For direct control of fruit pests, the concentration must be sufficient to be effective against the pest while ensuring relatively low residues in the fruit at harvest.

7. Conclusion and future research needs

Trunk injection could thus be a valuable alternative to spraying, particularly to reduce the use of pesticides. Tree injection could be workable when traditional methods, such as soil and foliar applications, are restricted, difficult or ineffective. Trunk injection reduces farm workers' exposure to agrochemicals as well as risks for the environment. Trunk injection avoids drifting of plant protection products, leaf wash off, biotic and abiotic degradation, such as microbial or photochemical degradation, at the leaf surface. By reducing losses of plant protection products, trunk injection is expected to reduce the dose required in comparison with that required for spray applications. However, this could be counteracted by metabolism of the plant protection product in the tree.

There is a need for further research to better deliver efficient product concentrations to target sites. The main challenge is identifying homogeneous concentrations in trees to achieve optimum efficiency while avoiding too weak concentrations in some parts of the canopy that could lead to the development of tolerant hotspots. This last point could be a limiting factor in the further development of trunk injection. However, much remains to be done to adapt the preparation of a wider range of active substances to this method, which has now fully demonstrated its relevance. It may also be useful to develop new compounds or to rehabilitate less lipophilic active substances that move more easily in the xylem. This is especially true for fungicides that generally require more complete leaf coverage than insecticides. Other investigations are needed to determine the most efficient number of injection points for each tree species and each trunk diameter, but probably also to pests or pathogens of interest.

The last technical point is the need to insure the injection date, which is specific to each substance, coincides with the period during which the substance is required to act. This is indisputably the most difficult challenge to meet because of the time needed for the compound to be distributed within the tree. This adds an additional parameter compared to the optimization of a foliar treatment.

Finally, trunk injection for isolate trees can result in saving time and money by reducing the number of application and the dosage compared to conventional spray application. However, in fruit production, trunk injection requires time and labour due to the high tree density, which can result in an increase in cost. Economic studies are needed to show that trunk injection can be competitive in commercial production context.

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