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Application note

Application of tracking implants in grape hybrids: Adjustments to production practices and new health-compliant methodologies

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ABSTRACT

In order to adapt tracking implants in grapevine to production practices, four rootstocks belonging to two common *Vitis* hybrids [*V. berlandieri* × *V. riparia* (420 A, Kober 5BB, SO4) and *V. berlandieri* × *V. rupestris* (1103 Paulsen)] were tagged with radio frequency tags using the available methods: direct drilling of the pith from the distal cut of the rootstock or a "U" cut performed laterally on the rootstock below the grafting point. Tests were also combined with hot water treatments against phytoplasmas or applied to one-year-old grafted rootlings ready for transplantation in the vineyard to reduce tagging costs. In addition, novel health-compliant methodologies for ultra-high frequency (UHF) tagging were evaluated. To assess the effects of tag implantation in rootstocks, plant viability, functional vascular tissue area and tag reliability were calculated, as well as the effects of phytopathogenic fungi on wounds produced by tagging. The tagging procedure did not cause significant effects on viability and functional vascular tissue area. Tag reliability was set at more than 96%. Fungal infections caused less than 1% of infected vascular tissue area and tagging methods could be integrated with hot water treatments against phytoplasmas. Tracking implants were applied successfully to one-year-old rootlings that were ready for transplantation, even if tag reliability decreased. Novel semi-internal implants of UHF tags did not cause concerns about plant health but tags were exposed to environmental stress or fortuitous damage during farming practices.

1. Introduction

Nowadays, many foods and agricultural products have to carry identifying labels or documents, as required by law (e.g. 2000/13/EC), to establish a safe traceability system. In the EU, grapevine propagation materials in the certified category must respect the most recent directive (2005/43/CE) and associated labels have to report essential data such as the nursery where they were produced. Plant traceability, as in food production, can be supported by information technology (IT) and can be considered a best practice in agriculture, as is the case for livestock (Stumbos, 2005; Voulodimos et al., 2010). The IT revolution, exemplified by the Internet, has made traceability and monitoring economically feasible, permitting food products to be traced as they move through the labyrinth of the agricultural product supply chain. With regard to food plants, the application of IT solutions to keep track of the plant-to-food chain seems to be possible only in woody fruit species (Bowman, 2005; Ampatzidis and Vougioukas, 2009: Porto et al., 2011), including grapevine (Luvisi et al., 2010, 2013), while labeling and/or tracking of herbaceous plants presents difficulties. The current cost of microchips may represent the main limitation for their use in woody plants. However, in light of the high value of plants such as woody perennials, the most common target for sanitary certification, the cost may now be affordable (Luvisi et al., 2012a). In contrast to the situation with livestock, where technology plays an important role with electronically labeled and checked animals, crop farms generally have a low level of computerization due to the costs involved and the lack of urgency to shift to a more in-depth traceability system (Luvisi et al., 2012b). However, available technology can satisfy various current needs.

Radio-frequency identification (RFID) tags, which have been widely tested in agriculture (Ruiz-Garcia and Lunadei, 2011), can represent a safe tool to identify plants that are protected by rights

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or subjected to specific regulations. Moreover, this technology can be efficiently integrated with mobile devices (Cunha et al., 2010) and digitalization of data relative to plants has already been used to monitor health, collect samples and retrieve sanitary information (Kumagai and Miller, 2006; Thrane, 2008). Tests in grapevine have involved the use of tags implanted within the pith of rootstocks and this technology, if appropriately supported by information management systems, can support health verifications and be a useful tool for managing risks related to environmental impacts of production systems, chemical residues and the worldwide spread of plant pathogens (Sørensen et al., 2010, 2011).

In grapevine, the available tagging methods were designed to tag vine cuttings before grafting and the subsequent callusing step in the nursery. Until now, tests have been carried out with low frequency stock tags in vine cuttings with 1103 Paulsen only (Luvisi et al., 2010). Moreover, the combination of tagging with hot water treatment against phytoplasmas (an increasing practice in Europe) or the application of tagging methods before transplanting to vineyard (with strong reduction of costs) have until now gone uninvestigated. Furthermore, methods for ultra-high frequency (UHF) tagging to improve readability are currently limited due to the use of tags that are not presently on the market, and the risk of damage to small pith rootstocks (Luvisi et al., 2013). Therefore, the development of new methods is desirable.

In this paper, we evaluate the effects of available tagging methods applied to four common grape rootstocks and their integration with hot water treatments. Cheaper tagging of grafted rootlings and new health-compliant tagging methodologies with low-cost tags are also evaluated.

2. Materials and methods

To evaluate the effect of internal tag implants on grape hybrids, procedures A and B, as described in Luvisi et al. (2010), were applied to four different rootstocks. Procedure A consists of microchip insertion after direct drilling of the pith from the distal cut of the rootstock just before grafting, followed by microchip localization below the grafting point (Fig. 1A). Procedure B consists in a "U" cut performed laterally on the rootstock below the grafting point, involving tissues from bark to pith; the microchip is then located inside the pith, and cut tissues are manually reassembled (Fig. 1B).

The low-frequency glass-tags involved in procedures A and B showed some limits, mainly with regard to reading distance: this feature can be improved by using more powerful scanners (Bowman, 2010) or tags operating at higher frequencies, such as UHF ones (Luvisi et al., 2013). However, available procedures to

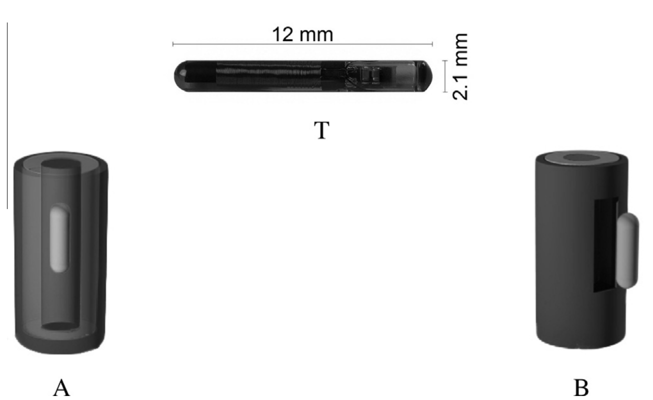

Fig. 1. Schemes of internal implanting in vine cuttings of LF tag (T) within the wound caused by: direct drilling of the pith from the distal cut of the rootstock (A); "U" cut performed laterally on the rootstock below the grafting point (B).

tag vines with UHF tags may cause significant damage in small pith diameter rootstocks, such as 1103 Paulsen. Furthermore, these methods do not allow for the use of currently marketed tags (Luvisi et al., 2013). In order to overcome these limitations, three semi-internal implants (SII) of UHF tags were tested. The tag was inserted within the wound caused by a side-cut (SII-A), within the wound caused by longitudinal cut of rootstock (SII-B), or within the wound caused by transversal drilling of the rootstock (SII-C), approximately 2.5 cm below grafting point (Fig. 2).

2.1. Internal tag implants

Procedure A was carried out on vine cuttings of *Vitis berlandieri* × *Vitis riparia* (420 A, Kober 5BB, SO4) and *V. berlandieri* × *Vitis rupestris* (1103 Paulsen) immediately before grafting them. Passive transponder glass tag RFIDs were employed (2.1 mm diameter and 12 mm length), working at a frequency of 125 kHz and with 256 bit of memory (InterMedia Sas, Forlì, Italy). Tags were electronically read using a wand reader (Livetrack RFID Wand Reader, Syscan-ID, Quebec, Canada) granting 10 cm in-air tag readability distance. To evaluate the effects of tag implantation in the rootstock, plant viability, functional vascular tissue area (VTA) and tag reliability were calculated. Viability was expressed as the number of viable plants out of the total produced, VTA was calculated by image analysis following Luvisi et al. (2013), and tag reliability was assessed as readable plants out of the total tagged plants.

Rootstock parameters were also evaluated after application of tagging procedures and other treatments that may cause wood stress. Hot water treatments (HWT) of dormant woody cuttings are used to control phytoplasmas (Boidron and Grenan, 1992) and in this study they were applied (50 °C for 45 min) before or after the tagging procedures on Kober 5BB or SO4.

Procedure B was carried out on the four rootstocks reported above, both on grafted cuttings (immediately after grafting) and on one-year-old grafted rootlings. Plant viability, VTA and tag reliability were assessed. In order to assure grapevine health, effects of phytopathogenic fungi on wounds possibly favored by rootstock tagging were evaluated following Marx et al. (2013). High inoculum concentrations of *Botrytis cinerea* and *Eutypa lata* were used. Fungal cultures were cultivated at 23 °C for 14 days on agar medium. Fungal mycelium was then scraped to collect spores which were added to water with Tween 2.0% to obtain the inoculation suspensions. The suspensions were dripped onto the wounded area of 30 tagged grafted cuttings (Procedure A or B) or on untreated vines grafted with the four rootstocks, replicating the inoculum treatment five times. The inoculated plant material was stored at 20 °C and relative humidity of 90% in order to simulate stressful storage conditions that enhance fungal infection (Marx et al., 2013). Fungal growth on the vine was evaluated after 14 days on fresh trunk sections in proximity to the inoculated area to determine the extent of the infected vascular tissue area (as necrosis or discolored tissue, I-VTA) of each sample. I-VTA was calculated using software for image analysis (ImageJ; National Institute of Health, Bethesda, MD), measuring the altered vascular area out of the total VTA, expressed as percentages.

Each experiment consisted of 30 tracking implants per rootstock and treatment.

2.2. Semi-internal tag implants

Semi-internal implants of UHF tags were proposed on grafted cuttings with Kober 5BB and 1103 Paulsen as rootstocks. Passive UHF tags (8.1 mm height and 94.8 mm length) were employed, working at a frequency of 840–960 MHz and with 512 bit of memory (Higgs, Alien Technology, Morgan Hill, CA). Tags were electronically read using a USB reader (Kenetics Group Lmt, St. Helier, UK)