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Propagative material of grapevine: RFID technology for supporting traceability of "basic" and "certified" material along the wine production chain

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Abstract: Four main categories of propagative material in the European Union (EU) have been indentified for grapevine: the primary source, pre-basic, basic and certified material. Each type of material has to be periodically assayed for pathogens and each material stage is intrinsically characterized by increasing risks. Radiofrequency (RFID) can be considered an efficient and durable traceability system to provide retrieval of propagated material or check producer identity. RFID tagging of grapevines of different categories along the production line could establish a durable link between stakeholder and products. To evaluate this approach, histological observations and growth parameters of basic or certified RFID-tagged grapevine were performed, as well as requirement analysis for management of sanitary checks and for traceability of the wine production chain. Basic material can be safety tagged with RFID tags to establish mother plant vineyards; derived certified material can also safely be tagged. No detrimental performance in terms of vegetative growth and bud production were reported for mother plant vineyards from the first year of production life. Requirement analysis made it possible to individuate traceability objectives, materials and stakeholders involved, as well as the RFID-tagging steps and methods to collect sanitary and production data that are useful for traceability purposes.

1. Introduction

Supply-chain traceability systems are suitable tools for controlling plant disease diffusion and they can be implemented by means of integrated computer-based information systems (ICBISs) which incorporate data from different production centers (Porto *et al.*, 2011).

With regard to propagative material for grapevine in the European Union (2005/43/CE), there are four main categories, labeled with a color coding system. The primary source, derived from selected grapevine by a conservative breeder and is grown under his/her responsibility: this source produces pre-basic material, as well as the derived basic material. This material is intended for delivery only to nurseries having the necessary qualification; certified grapevines are derived from this source. Italian regulations (DM 8 February 2005, DM 7 July 2006 and DM 24 June 2008) define which propagative material must be periodically assayed for pests: each material stage is intrinsically characterized by increasing risks of re-infection, and rapid and safe retrieval of mother plant data can be useful for prompt intervention when it is necessary to limit spread of the pathogen.

Surely an efficient and durable traceability system can provide for information retrieval regarding propagated material, particularly if it is supported by an information technology (IT) solution: radiofrequency (RFID)-based technologies can be implemented in platforms to share and manage data in agriculture, providing a safe and durable link between items - such as plants - and information (Sørensen *et al.*, 2010), with positive effects on traceability. Sørensen *et al.* (2011) stated that communication and automated processing of data require a digital form and a machine-readable format which can be interpreted unambiguously by all entities involved in the information flows. Increasingly, common data sources and sensor systems in agriculture produce digital, machine-readable data which can be used for decision making. With regard to grapevine propagative material, RFID technology has been successfully used to identify all plants during

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sanitary, ampelographic and genetic checks during selection, with no losses in retrieving information from plants (Pagano *et al.*, 2010). Grapevine tagging with a RFID microchip (tag) can be performed internally (Bandinelli *et al.*, 2009) in order to guarantee a durable identification of plants: a microchip identification code can also be associated to an electronic identification datasheet (eID) to store virtually unlimited data for each microchip/plant (Luvisi *et al.*, 2010 a). In this perspective, RFID tagging of grapevines of different categories belonging to a production line could establish a durable link between stakeholder and products: interestingly, this application could be combined with corks containing a RFID inlay for high-value wines (Collins, 2005; Launois, 2008). Histological observations and growth parameters were performed in four-year-old RFID-tagged basic plants to evaluate plant response to implanting method at production stage: tests were also performed on one-year-old derived plants, the information from which was linked to mother plants. Requirement analyses for plant management were also performed, while the wine production chain was described in order to define a traceability scheme based on RFID microchips linked to each production stage, from primary source registration to bottle tagging.

2. Materials and Methods

Plant materials

In order to evaluate the traceability procedures from foundation block to grower, pre-basic *Vitis vinifera* cv. Sangiovese were externally tagged with a RFID wristband. Then, derivate *V. vinifera* cv. Sangiovese cuttings was grafted on rootstock 1103 Paulsen (*Vitis berlandieri* x *Vitis rupestris*) by a foundation block in 2007, in order to obtain two rows of basic material. Hardwood cuttings were obtained from these plants in 2009 and 2010, and used by a nursery to graft on rootstock 1103 Paulsen in 2010 and 2011 respectively, in order to obtain two rows of plants of certified material. Plants belonging to basic or certified categories were tagged with RFID tags, following the Luvisi *et al.* (2010 b) procedure: microchips were inserted inside pith of rootstock after 4-cm depth direct drilling of pith from a distal cut of rootstock just before omega grafting. The microchip was positioned 3 cm below grafting point. Untagged plants were used as control in order to evaluate production performances.

In order to evaluate the tagging system along the entire grapevine production chain, conservative breeder activities were included in the trial. To minimize experimental times, *V. vinifera* cv. Sangiovese plants involved in a clonal selection procedure started in 2007 were used as primary source of plant used for the foundation block. Similarly, barrels and wine bottles were marked with external tagging to simulate data matching with plants, thus completing the traceability system of the wine production chain using RFID.

Electronic materials and requirement analysis

Transponder glass RFID tags were used (diameter 2.1 mm, length 12 mm), working at a frequency of 125 KHz (InterMedia Sas, Forlì, Italy, www.RFID360.net). They were used for internal implanting or attached to plastic wristbands for the external tagging procedure. Tags were electronically read using a Card Flash reader connected by SD slot to a palm-PC (Dell Axim X51) able to identify the microchips from a distance of 100 mm. Software for managing data was Java™ and Adobe® Flex®. Tag reliability, as number of readable microchips out of the total, and accuracy, as number of readable microchips within 30 sec, were evaluated in implanted basic (four-year-old) or certified (one-year-old) grapevine plants.

Requirement analysis was performed by stakeholder identification, interviews, collecting data relative to regulations, farm rules and existing software for managing RFID-tagged plants. According to Porto *et al.* (2011), the requirement analysis step regarded the a) identification of traceability objectives; b) analysis of the regulatory requirements concerning grapevine production, c) identification of plant propagating materials produced along the grapevine production chain; d) definition of RFID-tagged materials; e) analysis of the material flows within the plant supply chain; and f) analysis of the information to be managed in the traceability system.

Plant assay

In order to evaluate the productivity of RFID-tagged basic plants, growth of branches expressed mean relative growth rate (MRGR, mg d^{-1}) with one sampling period of 45 or 90 days was calculated from when shoots started growing in 2009 and 2010. In addition, mean number of buds recovered for grafting and mean diameter of buds were evaluated at the same time. Considering these parameters, mean data from 20 plants per treatment are reported.

To estimate long-term damage or losses in productivity, effects of implanting procedures on functional vascular tissue area (%) were measured at three heights in RFID-tagged basic (four-year-old) or certified (one-year-old) grapevine. Measurements were performed on fresh trunk sections in proximity of the microchip location, at approximately mid-length of the microchip ("height 0"), 3 mm higher ("height 3") and 3 mm lower ("height -3"). In unmarked plants, sections were taken at the same height as in marked plants. Vascular tissue area was calculated using software for image analysis (Cerri *et al.*, 1993), measuring total vascular tissue area and non-necrotic vascular tissue area. For histological observations, fresh transversal sections (20 µm thick) were made with a rotary microtome (Reichert-Jung, Autocut 2040, Austria) and stained with Toluidine Blue O (Sigma-Aldrich Corporation, USA); sections were immediately observed with a light microscope (Leica, Wetzlar, Germany).

Data analysis

The effects of treatments were compared by analysis of variance in a random design. Duncan's multiple range test at 5% level was calculated in order to compare treatments for functional vascular area, characterized by undamaged vessels, and in which xylem rays are developed as control, and for growth parameters as well. Data in percentage were normalized by arc sin square root transformation.

3. Results

Requirement analysis and microchip tests

The objectives for traceability of requirement analysis were: to record genetic links among plant categories; to record mandatory or voluntary assays; to identify structures or people involved in plant production and resulting wine; and to use an identification system which relies on RFID codes associated with electronic identity cards (Luvisi *et al.*, 2010 a). Regulations concerning non standard grapevine production require mandatory assay for conservative breeders or foundation stock (2005/43/CE). Data relative to assays, in particular periodical health assays, can be associated to tags through the use of a database. In any case, other checks, for example those performed by phytosanitary services or those for voluntary certification, can be implemented within the system. Identification of plant propagation materials produced along the grapevine production chain led to the creation of six plant categories to be included in the plant management system (Fig. 1): plants belonging to these categories can be tagged dif-

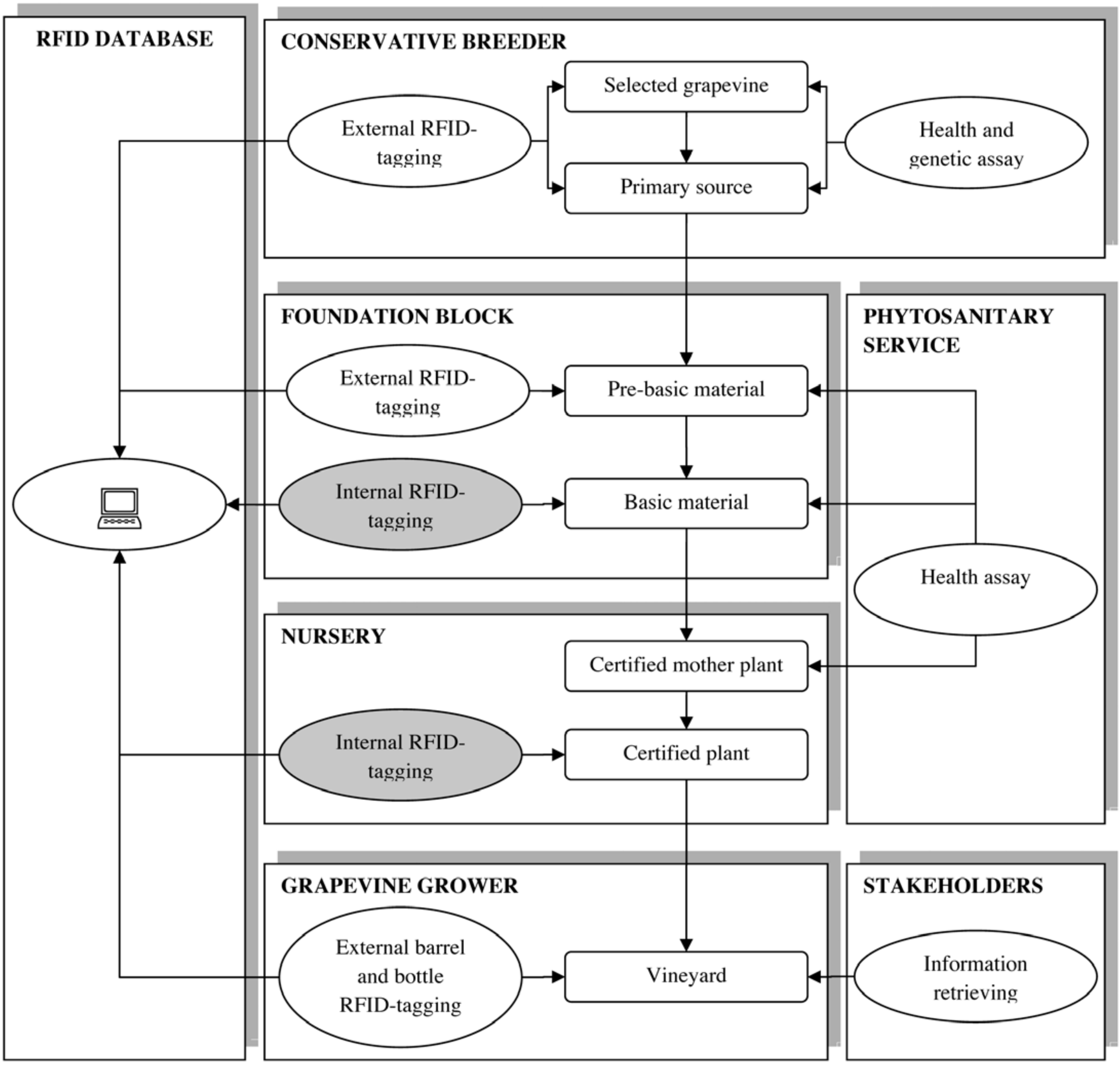

Fig. 1 - Data flow within RFID-tagged grapevine production chain.

ferently in relation to their morphological characteristics. External tagging is for grapevines selected by conservative breeders, primary sources and non grafted pre-basic material: these plants cannot be tagged using methods proposed thus far. Taking into account trunk dimension (which in the case of older plants may be adequate), transversal drilling of trunk and microchip implanting may be possible. On the other hand, tags can be implanted in basic and certified material by foundation blocks and nurseries, respectively. Furthermore, to establish a link with the final product, barrels and bottles can be externally tagged. Figure 1 offers a scheme to analyze the material flows within the plant supply chain. Analysis of the information to be managed in the traceability system identifies the information as: information from grapevine cultivar registries (i.e. the Italian *Registro nazionale delle varietà di vite*); information useful for grapevine purchasers, such as botanical name and cultivar, plant category, clone and rootstock specifications, name of producer, RFID-related codes; product history, such as the conservative breeder, mandatory or voluntary assays, information about stakeholders; a digital map accessible via mobile or desktop systems, providing a "virtual vineyard" in which grapevine plants marked by RFID can be viewed (Luvisi *et al.*, 2011); and details of wine production, wine producer and cellar.

With regard to tests of microchip reliability, tags were fully functional in both plant categories and the implanting procedures did not compromise readability. Tags were readable within 30 s in more than 98% of the reading tests, without significant differences between plant categories (Table 1).

Table 1 - Microchip reliability (as number of readable microchips out of the total) and accuracy (as number of readable microchips within 30 s), implanted in basic (four-year-old) or certified (one-year-old) grapevine plants

Grapevine category	Microchip reliability	Microchip accuracy
Basic	100.0 a (z)	98.8 a
Certified	100.0 a	98.1 a

(z) Within each parameter, values in the same column followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).

Plant assay

RFID-tagged grapevines belonging to the basic category did not show detrimental effects for parameters affecting their productivities such as growth and bud production. In fact, after two years from tagging, no differences in MRGR at 45 or 90 days were measured compared to control (Table 2). RFID-tagged plants develop a comparable number of buds with adequate diameters compared to control (Table 3). The absence of detrimental effects of RFID implanting in both categories was confirmed by measurement of the functional vascular tissue area, not affected by tag presence (Table 4).

Table 2 - Growth of branches of RFID-tagged basic grapevine expressed as mean relative growth rate (MRGR, mg d^{-1}) with one sampling period of 45 or 90 days, calculated from when shoots started growing

Year	MRGR (mg d^{-1}) 45 days		MRGR (mg d^{-1}) 90 days	
	Control	RFID-tagged	Control	RFID-tagged
2009	0.095 a (z)	0.100 a	0.121 a	0.125 a
2010	0.0101 a	0.099 a	0.128 a	0.126 a

(z) Within each parameter, values in the same line followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).
Mean data from 20 plants per treatment are reported.

Table 3 - Mean number of buds for RFID-tagged basic grapevine recovered for grafting and mean diameter of buds

Year	Mean No. of buds/plant		Mean diameter of buds (mm)	
	Control	RFID-tagged	Control	RFID-tagged
2009	27.3 a (z)	25.1 a	8.5 a	9.1 a
2010	29.4 a	32.5 a	10.8 a	10.0 a

(z) Within each parameter, values in the same line followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).
Mean data from 20 plants per treatment are reported.

Table 4 - Effects of implantation procedures on functional vascular tissue area (%), measured at three heights, in RFID-tagged basic (four-year-old) or certified (one-year-old) grapevine

Procedure	Functional vascular tissue area (%)					
	Height -3		Height 0		Height 3	
	Basic	Certified	Basic	Certified	Basic	Certified
Control	99.1 a (z)	98.7 a	99.9 a	99.4 a	99.8 a	99.0 a
RFID-tagged	99.2 a	99.1 a	99.8 a	98.7 a	99.9 a	98.8 a

(z) Values in the same column followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).
Mean data from 20 plants per treatment are reported.

4. Discussion and Conclusions

Plant labeling represents a key step in the certification scheme, with labels supplied by certifying authorities such as a government agency or an officially recognized private organization. For various woody plants, labeling is strictly regulated by law, and currently electronic identification is not an viable option for substituting plastic labels. However, interest in these tools by organizations such as the European Commission cannot be ignored, as reported for bovines, already in use in several EU member states on a private basis mainly for farm management purposes. This approach seems to be crucial for localizing and tracing individual animals for veterinary purposes as a tool to control infectious diseases (European Commission, 2011).

Pets can also be legally subjected to microchip implanting, as reported for dogs in Italy (Ordinanza 6 Agosto 2008). Even if plants cause less concern about human health when compared to the meat production chain, implications do exist in terms of worldwide spread of plant pathogens (in particular viruses) and chemical residuals. Plant traceability by RFID can be a useful tool when it comes to risk management.

Considering the grapevine production line, basic material can be safely tagged with RFID tags to establish mother plant vineyards, the same for derived certified material. No detrimental performance in terms of vegetative growth and bud production were reported for mother plant vineyards from the first phase of production life. Tags were readable to check identities after four years from implanting. Data associated to basic material can be linked to previous categories such as pre-basic and primary sources that can be externally tagged, completing the traceability of the grapevine production chain. Requirement analysis made it possible to individuate traceability objectives, materials and stakeholders involved, as well as RFID-tagging steps and methods to collect information and match data, from the plant to the wine bottle. Even if the availability of user-friendly details about wine production may seem sufficient for the final user (i.e. details about the cellar or viewing of virtual vineyards), the system guarantees retrieval of detailed, exhaustive data for inside users, thus supporting a virtuous circle of trust.

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