QUALIREDFRUITS

NEW AGRICULTURAL PRACTICES FOR QUALITY PRODUCTION OF RED FRUITS ENRICHED IN HEALTHY COMPOUNDS

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D02.04 Report for public dissemination on compatibility & synergy optimization of practices for biotised/elicitor treated raspberry

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SHORT DESCRIPTION as per the DoW: *This Deliverable D02.03 is a report on new culture practices for the production of raspberry based on biotisation and elicitor treatment to optimise fruit quality including the results on the compatibility and/or synergy between these cultural practices. The deliverable shall contribute to the final recommendations to the relevant SMEs.*

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Abstract

Previous project achievements showed that treatment of raspberry plants with plant defence elicitors can effectively reduce *Phytophthora* root rot and that biotisation with beneficial microorganisms can improve raspberry plant growth. Compatibility and synergy between the two treatments have been optimised under greenhouse and field production conditions and evaluated using appropriate indicators (mycorrhizal development, plant growth, defence-related enzyme activities, fruit production and AOM content/antioxidant activities in leaves and fruits).

Four tested elicitors showed compatibility with mycorrhiza development when applied during the standardized procedure developed for large scale production of biotised raspberry plants. One elicitor reducing root rot development independent of plant genotype was retained for detailed investigations under greenhouse and field production conditions. Under greenhouse conditions, beneficial effects of biotisation on flowering and early fruit production were maintained in elicitor-treated plants. Activity of some defence-related enzymes was modified in leaves of elicitor-treated plants, whether they were biotised or not; foliar application of the elicitor showed a systemic effect on enzyme activities in roots. Organic farming conditions enhanced compatibility in elicitor-treated, biotised plants with improvement of fruit size in a raspberry cultivar outplanted into a commercial cultivation system. This represents a promising step towards the development of innovative cultivation procedures respectful of the environment. However, biotisation and/or elicitor treatment did not consistently influence AOM content/antioxidant activities of raspberry leaves and fruits under greenhouse conditions or of fruits under field conditions.

Recommendations can be given to SMEs of procedures for the successful utilisation in raspberry production of compatibility between biotisation and elicitor treatment for plant growth and fruit yield under greenhouse and field production conditions.

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1. REMINDING THE MAIN OBJECTIVES OF THE PROJECT

The **Concept of the QualiRedFruits** project deals with the competitiveness of raspberry production and market.

Raspberry is a horticultural crop with a high value due to:
- ✓ Its unique organoleptic qualities,
- ✓ Its proven “health promotion – disease prevention” (HPDP) properties and,
- ✓ the high but still increasing competition between production areas worldwide.

In this context, this project is meant to provide a consistent combination of SME’s with the necessary research activities aiming at designing new raspberry varieties offering competitive advantages based on a high content in HPDP compounds while requesting cultural conditions, such as biotisation with beneficial micro-organisms (arbuscular mycorrhizal fungi and beneficial bacteria) and elicitor treatment, suited to environmental management.

The **Objectives of the QualiRedFruits** project is to meet the 5 following challenges:
- ✓ Broaden the marketing offer by introducing on the market new varieties originating from Europe;
- ✓ Offering such European varieties with a standardised assessment of their content in bioactive compounds as the role of these compounds in HPDP is increasingly established by medical research worldwide;
- ✓ Offering varieties that require significantly reduced chemical treatments as the result of innovating cultivation procedures respectful of the environment;
- ✓ Orienting the relevant regulatory framework towards the establishment of certification procedures based on easy genotyping of the new varieties and easy assessment of the sanitary status of the plants;
- ✓ Forging arguments to promote the informative labelling of the marketed fruits and processed derivatives concerning their HPDP properties including measurement of AOM content.

The **ultimate goal of the QualiRedFruits** project, beyond its completion, is to create competitive advantages of European stakeholders based upon enhanced quality, hygiene, food safety, nutrition, labeling and environmental-friendliness. Two major directions for improvement have been identified as (i) **the content in antioxidant molecules (AOM)** and (ii) the **tolerance to pathogenic fungi**.

2. BACKGROUND TO THE WORK

Biotisation is a biotechnology consisting in the inoculation of young plants with beneficial micro-organisms (bacteria and/or fungi) in order to increase plant survival and growth (Gianinazzi et al. 2003). Arbuscular mycorrhizal (AM) fungi, which are at the interface between plant roots and soil, are the most significant of beneficial soil micro-organisms as they establish a symbiosis with roots of most high value agricultural and horticultural plants, including raspberry. Other beneficial micro-organisms used in biotisation include bacteria such as *Pseudomonas* and *Bacillus* (Avis et al. 2008). AM fungi form an abundant mycelium in the soil, absorb relatively immobile mineral nutrients such as phosphorus and transfer them to their host plant in exchange for carbon compounds. This results in a modification in root system morphology and in plant physiology, which is in favour of the development of beneficial micro-organisms in the rhizosphere and limits pathogenic micro-organism development (Gianinazzi et al. 2010). Mycorrhizal plants become more vigorous and more tolerant to biotic and abiotic stresses. In the case of raspberry, biotisation of young plants during the acclimatisation phase has proved to be useful in raspberry for improving plant survival and growth and increasing tolerance to *P. fragariae* var *rubi* (Gollotte et al. 2009, Lemoine et al. 2000). Although AM fungi are present in most ecosystems, their populations are generally limited in the field under conventional agriculture because of their sensitivity to practices using large quantities of pesticides and fertilisers. Moreover, micropropagated plants and substrates used in horticulture to grow plants are usually devoid of AM fungi. Biotisation can therefore be very useful to the plant in such cases. Biotisation can also be beneficial to plant growth and health in the field in organic farming or when reduced amounts of fertilisers and pesticides are used.

Elicitors of natural plant defence mechanisms have been shown to provide useful disease control in many crops against a range of economically important pathogens (Walters, 2011). Fungal diseases have the highest potential to cause serious raspberry yield loss. Wet and heavy soils or excess irrigation can enhance root rot due to *Phytophthora fragariae* var. *rubi*, the main cause of raspberry plant death. Chemicals do not effectively control this fungus and only prophylactic measures can be adopted by planting healthy raspberry plants in non-contaminated soils. For raspberry, breeding programmes have enabled the creation of a few cultivars which are more tolerant to *P. fragariae* var. *rubi*. Nevertheless, fighting against cryptogamic diseases is still a problem in raspberry cultivation. Using elicitors of plant defence reactions is a new alternative to chemical fungicides which can be pejorative to the environment and human health. It is well known that treatment of plants with various agents (e.g. cell wall fragments, plant extracts) can lead to the induction of resistance to subsequent pathogen attacks, both locally and systemically (Walters et al. 2005, Walters & Heil 2007). This induced resistance does not lead to complete disease control, but instead leads to a reduction in pathogen development and disease symptoms. Importantly, the expression of this induced resistance does not require the presence of major pathogen-specific resistance genes, although the defence mechanisms activated are those used in other forms of plant resistance to pathogens. The defences activated are numerous and include enhanced defence-related enzyme activities as well as synthesis and accumulation of a range of phenolic compounds, many of which possess antioxidant properties. The prospect of broad-spectrum disease control using the plant’s own resistance mechanisms has led to increasing interest in the development of agents which can mimic natural inducers of resistance (Walters & Heil 2007).

The AOM concept refers mainly to non-nutrient compounds in foods and covers a broad cultivar of molecules including flavonoid or non-flavonoid polyphenolics, phenolic acids, vitamins and other potential organic antioxidants (Beekwilder et al. 2005). Raspberries rank near the top of all fruits for antioxidant strength, but most red raspberry cultivars presently on the market contain relatively low levels of AOM compared to black raspberry, blackberry and wild raspberry. AOM synthesis in plants depends on several factors including plant cultivars, climatic conditions and agricultural practices (Anttonen et al. 2005). It has been shown, for example in strawberry, that reduced fertilisation regime resulted in an increase in AOM content (Anttonen et al. 2006). However, the impact of agricultural practices on plant content in AOM has not been characterised in the case of raspberry. Mycorrhization has been reported to increase polyphenol content in grapevine and sweet basil, carotenoid level in sweet potato, and antioxidant activity in raspberry (Krishna et al. 2005, Farmer et al. 2007, Gollotte et al. 2009). Also, treatment with plant defence elicitors has been reported to increase total phenolic content and antioxidant activity in sweet basil (Kim et al. 2005), and to increase total phenolic, anthocyanin and antioxidant content raspberries and blackberries when applied during the ripening phase (Wang & Lin 2000, Wang & Zheng 2005, Wang et al. 2008).

Knowledge about compatibility, or synergy, of plant defence elicitors with biotisation in raspberry plant production systems is so far inexistent. In plants other than raspberry, positive effects or no effect of elicitor treatment have been observed on the colonisation of roots by AM fungi (Murphy et al. 2000, Sonnemann et al. 2000, Tosi & Zazzerini 2000, Hause et al. 2007). The enhancement and the consequent standardization of raspberry plant biotisation coupled with elicitor treatment are expected to be of competitive advantage for the SMEs in terms of healthier plant production and fruit quality.

3. MAIN RESULTS AND CONCLUSIONS

The work was aimed at optimising the association of biotisation and elicitor treatment for introduction into raspberry production systems to promote decreased chemical inputs and increased AOM synthesis. The objectives have been attained through the combined use of (i) AMF isolates and beneficial bacteria selected for their efficiency and compatibility, (ii) an optimized procedure for biotisation of raspberry plants before outplanting, and (iii) effective elicitor treatment for reducing development of *Phytophthora* root rot symptoms in raspberry plants. Compatibility between biotisation and elicitor treatment was evaluated under greenhouse and organic farming conditions using appropriate indicators: mycorrhizal development, plant growth, defence-related enzyme activities, fruit production and AOM content/antioxidant activities in leaves and fruits.

3.1 Compatibility and synergy between elicitor treatment and biotisation of raspberry plants under greenhouse conditions

During large-scale plant production

Four elicitors which can reduce *Phytophthora* root rot in raspberry plants were compared by P06-INRA for effects on mycorrhizal development and plant growth during the large scale greenhouse production of biotised raspberry plants. Elicitors were applied as an aerial spray at two week intervals after transplantation of non-biotised or biotised plants of an *in vitro* propagated raspberry cultivar into 1l pots and effects monitored up to 8 weeks. All four elicitors were compatible with biotisation. Although mycorrhizal development decreased slightly following a second elicitor treatment, levels subsequently increased to reach those comparable to plants not treated with the elicitors. None of the elicitors altered the beneficial effect of biotisation on raspberry plant growth which remained greater than in elicitor-treated, non-biotised plants.

One elicitor was retained for further detailed investigations of effects on defence-related enzyme activities, fruit production and AOM content/antioxidant activities in leaves and fruits of biotised plants produced under greenhouse conditions.

Root and leaf samples of the raspberry plants produced by P06-INRA were analysed by P07-SAC after the last elicitor application for the response of three enzymes known to be related to defence activation in other plants. Effects varied between the enzymes, plant parts and biotisation. Whilst two enzyme activities were decreased by the elicitor in the leaves of all plants whether they were biotised or not, two showed systemic responses to the elicitor with increased activities in the roots of elicitor-treated biotised plants.

Plants overwintered for fruit production

Biotised plants previously treated with an elicitor in 2011 were transferred into a fresh biotisation substrate and overwintered in an unheated, unlighted greenhouse for fruit harvest and AOM analyses in 2012; non-biotised control plants were transferred into a non-biotised substrate. The elicitor was foliar applied again at early and late flowering stages.

Mycorrhizal colonization was unaffected by the overwintering period in biotised plants, treated or not with the elicitor, and biotised plants developed a higher number of buds and flowers than non-biotised ones in spring. During the first five weeks of harvest, total fruit yield and number were greater in biotised compared to non-biotised plants with values for yield ranging from +37% in elicitor-treated biotised plants to +60% in absence of the elicitor. At the last harvest after 9 weeks, no differences were observed between treatments.

Frozen fruits and healthy terminal leaflets from the overwintered raspberry plants were extracted for quantification of AOM content and antioxidant activity by P08-ACW. The leaflets of non-biotised plants treated with the elicitor showed an increase in some AOM and antioxidant activities compared to the other treatments. This was not the case for the biotised plants where levels of AOM and antioxidant activities decreased in leaves as compared to non-biotised plants, a trend which was accentuated by elicitor treatment.

Different observations could be made for the fruits where elicitor application tended to AOM and antioxidant activities in non-biotised plants. In plants not treated with the elicitor, biotisation had little effect on these fruit qualities. In biotised plants treated with the elicitor, on the contrary, the levels of AOM, but not antioxidant activities, were higher in the fruits as compared to elicitor-treated non-biotised plants.

Conclusions

Foliar applied plant defence elicitors are compatible with biotisation in raspberry plants under greenhouse production conditions. No detrimental effect is observed on root colonisation by the AM fungus used in biotisation or on raspberry plant development, and beneficial effects of biotisation on flowering and early fruit production are maintained in the elicitor-treated raspberry plants. Elicitor effects on defence-related enzyme activities differ between plant parts, and systemic responses are enhanced by biotisation in roots. The influence of biotisation and/or elicitor treatment on AOM content and antioxydant activities is not consistent between leaves and fruits.

3.2. Compatibility between elicitor treatment and biotisation under organic farming conditions

A field trial was performed to evaluate the effects of biotisation and elicitor treatment on two raspberry cultivars in an organic production system. Biotised and non-biotised plants were produced on a large scale by P06-INRA, and planted out by P12-LPL into their field location. One or two plant-derived liquid manures were applied regularly after outplanting in the first year. Half the plants in each treatment were foliar treated or not with a plant defence elicitor at 4, 8 and 12 weeks after plantation in 2011 and at bud break, early and full flowering in 2012. Mycorrhizal development was monitored after elicitor treatment, and fruits of both cultivars were harvested in 2012.

Treatment with the elicitor had no negative effect on mycorrhizal development, whatever the raspberry cultivar or the liquid manure applications. Non-biotised plants quickly became mycorrhizal with indigenous fungi after outplanting. At 12 weeks after transplantation, there was a synergistic effect on mycorrhizal development for the two raspberry cultivars in treatments where they received the two liquid manures, and mycorrhizal levels were higher in the elicitor-treated plants compared to untreated plants whether they were biotised or not at outplanting. High levels of mycorrhization persisted in the second year after outplanting.

No significant variation was observed in the mean fresh weight of fruits from either raspberry cultivar between plants treated or not with the elicitor, whether they were biotised or not before outplanting. However, application of both liquid manures under organic farming conditions increased fruit size in previously biotised plants of one cultivar compared to those receiving only one, and this synergistic effect was accentuated in elicitor-treated plants.

Analyses of the harvested fruits by P08-ACW showed clear differences in AOM content and antioxidant activities between the two raspberry cultivars. In plants treated with both liquid manures, the impact of biotisation and/or elicitor treatment on AOM contents and antioxidant capacities varied between the two raspberry cultivars, having no effect in one cultivar, across treatments, and decreasing slightly with elicitor treatment alone or combined with biotisation at outplanting.

Conclusions

A combination of biotisation and elicitor treatment can be successfully applied to raspberry plants outplanted into a commercial cultivation system. Moreover, elicitor treatment and organic farming conditions are compatible with mycorrhiza development and synergistic beneficial effects on fruit size can be observed with liquid manure application. This represents a promising step towards the development of innovative cultivation procedures respectful of the environment. AOM production and antioxidant capacities of fruits do not appear to be clearly influenced by biotisation and/or elicitor treatment. The lack of any observable interactions between biotisation and elicitor in terms may be linked to the fact that the non-biotised plants also develop mycorrhiza quickly with indigenous fungi after outplanting.

3. GENERAL CONCLUSIONS

All the tested plant defence elicitors have shown compatibility with biotisation when applied during the standardized procedure developed for large scale production of biotised raspberry plants. Under greenhouse conditions, compatibility is reflected by synergistic effects on mycorrhiza development, some defence enzyme activities and fruit production. Organic farming conditions enhanced compatibility in elicitor-treated, biotised plants with improvement of fruit size in a raspberry cultivar outplanted into a commercial cultivation system. However, neither biotisation nor elicitor treatment show a clear and consistent impact on the AOM content or antioxydant capacities of the raspberry fruits or the leaves.
Recommendations with defined protocols can be made to SMEs for the successful utilisation in raspberry production of both biotisation to improve plant growth/fruit yield and plant defence elicitors which effectively reduce *Phytophthora* root rot.

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