ISSN: 0394-6169
ISNN: 1592-1573

ADVANCES IN HORTICULTURAL SCIENCE

n. 1 **2012**

formerly
«Rivista dell'Ortoflorofrutticoltura Italiana»
founded in 1876

FIRENZE UNIVERSITY PRESS

Advances in Horticultural Science
Published by **Firenze University Press** - University of Florence, Italy
Via Cittadella, 7 - 50144 Florence - Italy
http://www.fupress.com/ahs

Direttore Responsabile: **Franco Scaramuzzi**, University of Florence, Italy.

ADVANCES IN HORTICULTURAL SCIENCE

Formerly Rivista dell'Ortoflorofrutticoltura Italiana
founded in 1876 and issued by University of Florence, Italy

supported by ENTE CASSA DI RISPARMIO DI FIRENZE

Advances in Horticultural Science is covered in the following indexing and abstracting services: BIOBASE - Biological Abstracts - BIOSIS Previews - Horticultural Abstracts - Ornamental Horticulture - Plant Breeding Abstract

Advances in Horticultural Science is published by the Department of Agri-Food Production and Environmental Sciences, University of Florence, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.
Phone +39-055-4574021-22, Fax +39-055-4574078-17, E-mail: advances@dipsa.unifi.it, Homepage: http://www.dipsa.unifi.it.

SUBSCRIPTIONS - The subscription price of volume 26, 2012 is € 60. 00 in Italy and € 70.00 in other countries. Mailing costs: € 3 for Italy, € 6.50 for Europe and € 10.00 for the rest of the world. The subscription price of an issue is €17.00 in Italy and € 20.00 in other countries.

CONTENTS

Adv. Hort. Sci., 2012 26(1): 3-16

Aerated water irrigation (oxygation) benefits to pineapple yield, water use efficiency and crop health

J. Dhungel, S.P. Bhattarai[(1)], D.J. Midmore
Centre for Plant and Water Science, Faculty of Science Engineering and Health, Central Queensland University, Rockhampton, QLD 4702, Australia.

Key words: *Ananas comosus*, CAM photosynthesis, 'D' leaf, *Phytophthora,* root respiration.

Abstract: Pineapple roots need adequate oxygen to function, sustaining growth and yield. The crop is susceptible to soil saturation caused by natural rainfall or irrigation, or even with drip irrigation that creates sustained wetting fronts. Drip and subsurface drip irrigation can develop sustained wetting fronts, particularly in low permeability soils, predisposing plant roots to a low oxygen environment. We evaluated the use of aerated irrigation water "oxygation", employing Mazzei air injectors which mix air with irrigation (12% air by volume of water) in-line, increasing oxygen concentration in the irrigation water stream. The effect of this treatment was evident in growth, development, and leaf gas exchange parameters. Total fruit yield increased by 44 and 26% whereas industry yield increased by 11 and 6% due to oxygation compared to the control and no irrigation, respectively. High yield was associated with an increase in fruit size and not the number of fruits produced. *Phytophthora* infestation in the oxygation (3% of plants) was significantly reduced compared to the control (4.9%), and without irrigation treatment (10.5%) suggesting that reasonable management of *Phytophthora,* which is one of the major pathological problems for pineapple production in Australia and elsewhere, can be addressed through aerated water irrigation. Oxygation responses were mediated through root and soil processes involving greater root biomass, root respiration, increased microbial diversity and enhanced soil aeration status.

1. Introduction

World production of pineapple (*Ananas comosus* L.) reached 19 million tonnes in 2008 with the industry dominated by Brazil followed by Thailand, the Philippines and Indonesia. In Australia, pineapple, as an exotic species, is grown almost exclusively in Queensland, producing 104,000 tonnes annually with an industry average yield of 37.9 t ha^{-1} in ~2700 ha (Dhungel *et al.*, 2009) contributing an annual farm gate value of Au$50 million (FAO, 2011). Thanks to Crassulacean acid metabolism (CAM), pineapple is adapted to dry environments. However, pineapple is sensitive to water-logging and therefore requires a well-drained soil with good aeration when grown with irrigation. Pineapple response to irrigation is generally high for yield and quality.

Industry interest in developing irrigation for pineapple has been triggered by recurring episodes of drought brought about by climate change, and a major shift in the historical rainfall pattern, and under such circumstances strategic and supplementary drip irrigation are imperative for a sustainable industry (Camp *et al*., 1993). Increased cost of water, reduced ground water reserves and vocal public pressure have forced growers to look for more effective alternatives to the traditional surface flood and furrow irrigation. In response, increasing adoption of localized micro irrigation such as sprinkler, drip irrigation (DI) or subsurface drip irrigation (SDI) is taking place to improve water use efficiency (WUE) and to minimize environmental impacts by reducing runoff and deep drainage (Thompson *et al*., 2002).

The irrigation efficiency of sprinkler systems is less than for drip systems due to evaporative losses and the tendency to promote foliar diseases in the former. Although far more efficient, DI and SDI can induce temporal hypoxic conditions in the rhizosphere due to their sustained wetting fronts, particularly in fine textured soil (Machado *et al*., 2003) where oxygen may not be sufficiently available for root respiration. Localized water-logging purges the soil pores of air and causes hypoxia, reducing root metabolic activity and function (Goorahoo *et al.,* 2002; Bhattarai and Midmore, 2009). Alleviation of hypoxic rhizosphere conditions can be achieved through the use of aerated water for irrigation, increasing oxygen availability in the root zone (Su and Midmore 2006; Dogan *et al.*, 2008). Oxygation, the term we use for aerated irrigation water with SDI, has been shown to benefit growth of a range of crops, particularly in heavier soils. This is because even under normal irrigations, roots can suffer due to a lack of soil oxygen. An increase in crop yields and WUE has been

(1) Corresponding author: s.bhattarai@cqu.edu.au

Received for publication 8 April 2011
Accepted for publication 18 December 2011

confirmed by Bhattarai *et al.* (2004) with the use of aerated water irrigation in a number of annual crops with C_3 metabolism. Other studies on other crops in a range of soil types with drip and SDI have also been implicated in creating a wetting front that can induce the possibility of hypoxia in the rhizosphere (Goorahoo *et al*., 2002; Midmore *et al.*, 2006).

Drip irrigation which involves point source water application in the rhizosphere could lead to constantly poor aeration around the root zone and predispose pineapple plants to a number of physiological disorders and susceptibility to *Phytophthora* root rot. Oxygation, using aerated water that has potential to ameliorate the hypoxic/ anoxic conditions, may be of benefit not only to improve the WUE, yield and quality but also to minimize disease caused by *Phytophthora,* with symptoms of root and fruit rot in pineapple. Previous research in annual crops has demonstrated the potential of oxygation to improve yield and WUE (Bhattarai and Midmore, 2009).

Most of the effects of water-logging in pineapple are mediated through the roots, including predisposition of the root system to a number of diseases including root and fruit rot. Generally field grown pineapples tend to develop roots in the upper soil layer, where they are confined and follow the wetting area. As the root activities are bound to the wetting fronts, the susceptibility to *Phytophthora* is generally high. Recent adoption of drip irrigation for pineapple in Australia and elsewhere is encouraging. Introduction of aerated drip systems can be pivotal in improving irrigation water use efficiency and minimising the infestation by *Phytophthora* in pineapple. The main objective of this trial was to determine under what conditions there is a measurable positive effect of aeration and how that relates to impact upon incidence of *Phytophthora* and on general crop growth and development. The study was designed to evaluate the effectiveness of oxygation in a perennial crop - pineapple - with the CAM pathway for carbon fixation, evaluating the benefits on fruit yield, quality, and water use efficiency. We report the results of field research carried out in collaboration with Valley Syndicate Pineapple Farm in Yeppoon, QLD Australia from 2007 to 2011 in order to evaluate the above mentioned effects of aerated water for drip irrigation.

2. Materials and Methods

Trial site and soil

The field experiment was conducted at Valley Syndicate Pineapple Farm, Yeppoon, Central Queensland, Australia (23°9'31.12°S, 150°42'51.36°E). The crop was grown over the period 2007-2011 from which two harvests were taken as main crop and first ratoon crop. Total area of the experimental site was 2.15 ha on a calcareous sandy loam soil, with organic carbon 0.68-1.2%, total nitrogen 0.06-0.09%, potassium (Colwell) 25-139 mg/kg, and phosphorus (Colwell) content of 18-39 mg/kg. The crop seasons were relatively wet compared to long-term averages. The region is described as a semiarid tropical environment, with summer-dominant rainfall (Fig. 1).

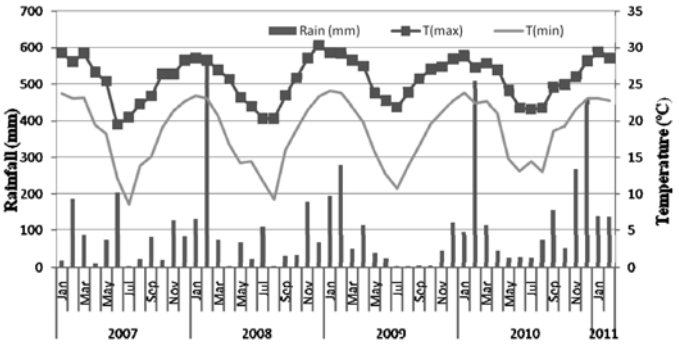


Fig. 1 - Total monthly rainfall (mm), monthly average maximum temperature (°C), and monthly minimum temperature (°C) over the experimental period at Yeppoon.

Experimental design

The experiment was a randomised block design, with two SDI irrigation treatments, with and without oxygation. Air injection into the irrigation water for oxygation was supplied by a 1583™ air injector venturi (Mazzei Corp, USA) installed in-line immediately before the field plot, regulated to ingress 12% air by volume of water following Bhattarai *et al.* (2006). The experiment was replicated seven times within 14 field plots (average dimensions 16 m x 70 m) where seven plots received aerated water by SDI and seven plots were irrigated without aerated water. An adjacent block, comprising three plots, received no irrigation (although all other inputs such as fertilizer, flowering regulation chemicals, fungicides and insecticides were identical) and was included as a third treatment (no-irrigation) for comparison purposes.

Planting materials and crop management

Crowns of pineapple variety GC1 were planted on 24 October 2007, in a double row, raised bed (10 cm high) system to accommodate up to 53,333 plants/ha. Inter row space was 50 cm and between plants was 25 cm, whereas centre to centre between the bed was maintained at 1.5 m. The oxygation treatment commenced 14 March 2008 (139 days after planting). Ethylene (ethephon) was applied twice as a saturated solution in water and activated charcoal to enhance absorption to induce flowering, as pressurized spray late in the evening or at night for enhanced uptake through the stomata. Plants were of an optimum size (~2 kg) for forcing in order to obtain even flowering.

Irrigation design and scheduling

Irrigation was blocked in four units. A Mazzei air injector was installed near the block (3 m from the first plot), whereas the pump was installed near to reservoir about 1 km distance from the plot. The inlet pressure of 45 PSI was achieved at the point of the Mazzei air injector installation. A pressure differential across the air injector was maintained at 45 and 15 PSI for the inlet and outlet, respectively, to maintain air injection at 12% by volume

of water (Fig. 2). The drip tube (manufactured by Plastro Australia) consisted of pressure compensated emitters at 30 cm intervals emitting at the rate of 1.2 l/hr, buried at 150 mm from the soil surface, one drip tube per bed running between two 50-80 m length crop rows.

Fig. 2 - Mazzei air injector installed in a pressurised irrigation line close to the plot for air injection into irrigation water for oxygation (top), and the first year crop at fruit development stage (b) being marked for destructive sampling.

Irrigation and soil water monitoring

Soil water content was monitored at depths of 10, 20, 30 and 40 cm, logged at 15 min intervals but averaged daily, using a calibrated Odyssey GreenLight-RedLight™ (GLRL) sensor (capacitance probes manufactured by Data Flow System Pvt Ltd, NZ). There was one monitoring site in each plot. Soil moisture was also measured (at 10 cm intervals from 10-60 cm depths) during the diurnal measurement of photosynthesis over a period of five days in January 2010; a calibrated Odyssey Micro-Gopher system, the probe of which consists of a capacitance sensor (Soil Moisture Technology, Australia) was used. Scheduling of irrigation was based on the averaged readings from the GLRL sensors at 20 cm depth; the amount applied was calculated to take soil moisture content when at C. <50% of the field capacity (FC 36 mm H_2O per 100 mm soil depth) to refill the soil water reservoir by irrigation to reach field capacity. This required different durations to bring the soil to FC.

Water applied to individual blocks (oxygation and control) was measured with two calibrated water meters installed near the pumping station and the rainfall data were accessed from a nearby weather station (<1 km aerial distance).

Nutrients to the crop were supplied through an industry standard rate of basal application of macro and micro nutrients and top dressing by spreading in the non-irrigated plot and by fertigation on drip irrigated plots supplied at the equivalent dose for all treatments. The industry strategies for increasing pineapple yield and fruit size included side dressing of nitrogen (160 k/ha), phosphorus (60 kg/ha) and potassium (193 kg/ha) over five split applications in a year. An additional dose of magnesium (12 kg/ha) was also applied once.

Soil oxygen monitoring

The O_2 concentration in the soil was measured at 15 cm depth between two emitters and offset 5 cm from the drip tube using PSt3 O_2 sensitive Fibre-optic minisensors with fibox-3 oxygen meters (PreSens GmbH, Germany) as described by Klimant *et al.* (1995). Sensors were installed in the soil for five days prior to data collection and soil oxygen monitoring took place for two days before oxygation, during oxygation and two days post oxygation event in the oxygation and control plots following the procedure of Chen *et al.* (2010). Soil oxygen monitoring was also performed in the ratoon crop over the period of four days before (-48 hr), during (0 h) and post (+48 hr) irrigation.

Destructive plant sampling for dry matter partitioning

Destructive plant sampling for dry matter accumulation and partitioning was carried out on 22 Nov 2008 (392 DAT) and 15 January 2010 (713 DAT) to evaluate the treatment effects in the main and ratoon crops. Plant samples collected from two whole 2 m linear lengths per bed were separated into leaf, stem, fruits and roots, oven-dried at 70°C, and the fresh and dry weights of each component were recorded. The 'D' leaf is always easy to pull from the plant and has leaf margins that are more-or-less parallel all the way to the leaf base (Bartholomew, 2008). The 'D' leaf is defined as the youngest physiologically mature leaf on the plant and also is the tallest leaf on the plant. Plant height and SLA were measured on the 'D'leaf.

Diurnal changes in gas exchange and plant parameters

Light interception by the canopy was measured using an AccuPAR Ceptometer (Decagon, USA) and canopy temperature was recorded using an Everest AG Multimeter. Leaf photosynthesis (A), transpiration (E) and stomatal conductance (SC) were measured at 6 hr intervals using an Infrared Gas Analyser (IRGA) LCA-4 (ADC, UK) on two fully-expanded topmost sunlit leaves per plot on each occasion at early morning (dawn), midday, dusk and at night following Adams *et al.* (2002). Soil respiration was

measured in the soil 3-5 cm from the plant using IRGA principle with an EGM-3 (PP Systems (UK) following Hanson *et al.* (2000) on 392 DAT for the main crop and 713 DAT for the ratoon crop. Data collection was also carried out to measure the gas exchange responses of the crop before, during and after oxygation events in the aerated SDI, the control SDI and no irrigation treatments. Pre-irrigation data were collected over a 24 hr period, on 14-15 January 2010 at 6 hr intervals to include dawn, midday, dusk/early evening and night to determine diurnal patterns of leaf gas exchange parameters during and post irrigation, and data on soil moisture and soil respiration were also collected using Microgoopher and EGM-3 soil respiration systems, respectively.

Harvesting and yield determination

Harvesting was performed at two scales, i.e. sample plot harvesting for total yield and industry harvest for marketable fruits yield. For sample plot harvests, all fruits were hand-picked at maturity from two rows of 2 m linear lengths (16 plants) selected from each of the seven replicated bordered plots, and from three plots in the non-irrigated area. The fruits were counted, weighted and processed for quality parameters for both main crop and ratoon crop. Change in fruit colour, particularly to the eye, from green to yellowish was considered an index for maturity and harvesting. Fruits without crown and peduncle were weighed. The industry harvest represents only marketable fruit yield harvested by the crew of pickers in a mobile harvester. Commercially-harvested fruits were weighted in the load cells with wooden crates containing ~500 kg. Commercial harvest was carried out for each plot separately at approximately weekly intervals. The main crop was harvested from January to April 2009 and it was left for ratoon which recommenced harvesting in 2010. The ratoon crop was harvested 28 June to 11 October 2010, and the whole crop was then uprooted for planting of a new crop, hence the crop spanned a period of 39 months from planting.

Fruit quality determination

Mature fruits harvested from sample areas were used for quality determination. Fruit quality parameters measured included °brix, fruit size, volume, density, fruit height and width, flesh colour, skin colour, dry matter, translucency and flavour, following the standard analytical method described by Bartolome *et al.* (1995). Fruit quality was assessed on fruits from different harvests in the main and also in the ratoon crop. In total, 20-50 randomly selected mature fruits were assessed for quality in the main and ratoon crops. The flavor score was determined based on smell by a panel group following industry standard; 1= no flavor, 2= little flavor, 3= good flavor.

Water use efficiency

Crop water use efficiency was calculated to represent irrigation water use efficiency (IWUE) and gross water use efficiency (GWUE). IWUE is calculated as fruit yield (t) per megaliter of irrigation input, whereas GWUE is the fruit yield (t) per megaliter of crop water input, comprising both the inputs from irrigation and rainfall. Instantaneous water use efficiency (WUE_i) was calculated from the IRGA data which represent μmol CO_2 assimilated for each mmol of H_2O transpired during measurement of the photosynthesis process.

Soil physical and chemical parameters

Changes in soil physical and chemical properties were assessed by measuring soil compaction, bulk density and air filled porosity (following the method of USDA, 2010) and soil organic carbon (Kuhlbusch, 1995) and nitrogen (ISO 13878 Soil quality - elemental analysis). Soil compaction was determined with Remek CP4011 soil cone penetrometers (ICT international, Australia) during the destructive plant sampling period to a depth of 35 cm from the soil surface. Soil water samples were collected at 50 cm depth for sub-surface leaching and nutrient analysis particularly nitrate signature using wetting front detectors (CSIRO, Australia). Subsurface solution samples were also collected from ceramic solusamplers placed at depths of 20 cm and 50 cm to determine nutrient transfer through the soil profile. At the end of the crop season, soil cores (20 cm deep and 8.6 cm diameter) were collected to determine bulk density, root density, air filled porosity at field saturation, field capacity and in dry soil following the method by Peverill *et al.* (2002).

Soil microbial and phytophthora determinations

Fluorescein diacetate hydrolysis activity (FDA) analysis provides a surrogate measure of the soil microbial load. Soil samples were collected during harvest at the depth 10 cm and 10 cm distance from emitter, and were used for FDA analysis following the method described by Adam and Duncan (2001). The incidence of *Phytophthora* infestation was assessed in the field crop. In each plot, a double row comprising ~500 plants was examined for infestation based on the visual symptoms following Pegg (1977). Plants with characteristic symptoms of *Phytophthora* on fruit and crown rot were counted and percentage infestation calculated.

Data analysis

Data were analysed following the procedures for analysis of variance (ANOVA) for randomised block design in GenStat Version 11 (VSN International, UK) as the no irrigation plots were contiguous to SDI plots. For most of the crop, only the main effects of soil and water parameters are presented, whereas for all other data collected during the diurnal events (leaf and soil gas exchange parameter) the effects of the treatment on the diurnal course were also analysed. Hence some significant interactions between the treatments and diurnal effects have been analysed and presented. Means were separated by the least significant difference (LSD) at $P \leq 0.05$.

3. Results and Discussion

Weather conditions

Rainfall was recorded as 917 mm, 1294 mm, 875 mm, and 1850 mm for 2007, 2008, 2009 and 2010, respective-

ly (Fig. 1). 2010 had the highest rainfall, more than four times that of the driest years in the past 20 years. Total rainfall input during the entire crop period was 4250 mm.

Irrigation input

Irrigation scheduling was based on measurement of the soil moisture deficit. The same delivery system through Mazzei injector was utilized for air injection and water application (Fig. 2). Irrigation commenced when soil moisture reached the refill point. Irrigation input during the crop period for the oxygation and control treatments was 252.4 and 240.5 mm per hectare respectively (Fig. 3). Rain contribution during the same period was 4250 mm per hectare (Fig. 1). Therefore, the proportion of irrigation to total crop water input was only 5.5%. All irrigation events were scheduled for supplementary and strategic applications to the crop.

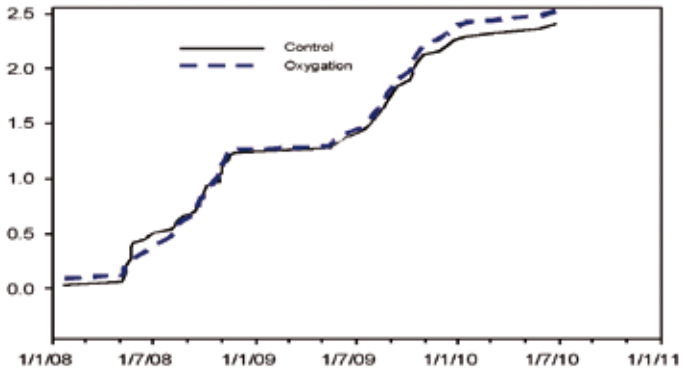


Fig. 3 - Cumulative irrigation input (ML ha^{-1}) over the crop period for oxygation and control treatments.

Soil moisture in the profile

Soil moisture at 40 cm was consistently higher than at 20 cm, irrespective of the treatment (Fig. 4).

Oxygation maintained somewhat less soil moisture compared to the control at both depths. On a number of occasions, the soil moisture content was well above the field capacity (36 mm for 100 mm soil depth) at greater depths, particularly in the control treatment. The results suggested that soil moisture increased with increasing depths (0-60 cm), irrespective of the treatment (Fig. 5). The effect of irrigation treatments on soil moisture was evident, as soil moisture always remained lower with oxygation compared to the control for all the depths in spite of slightly higher irrigation input associated with oxygation, suggesting that water loss from the rhizosphere was greater through transpiration for plants with the oxygation treatment.

Soil oxygen dynamics

Soil oxygen concentration in the oxygated rhizosphere remained higher (7.5 *vs* 4.4 ppm) than the control. The highest oxygen concentrations (10.72 and 7.08 ppm) were noted during irrigation for the oxygation and control groups, respectively. The lowest concentrations for oxygation and control were 5.48 and 3.09 ppm, respectively

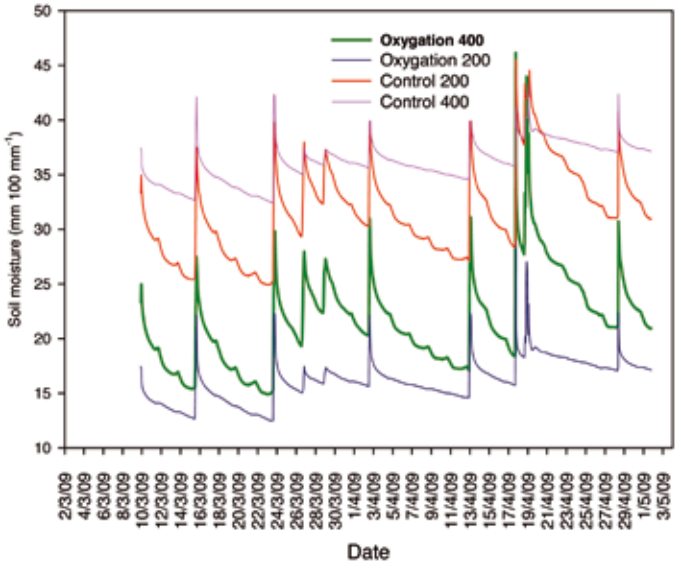


Fig. 4 - Change in soil moisture (mm water 100 mm^{-1} of soil depth) at two different depths (400 and 200 mm) over the period of two months (March-April 2009) in the oxygation and control treatments measured by Red Light Green Light soil moisture sensors.

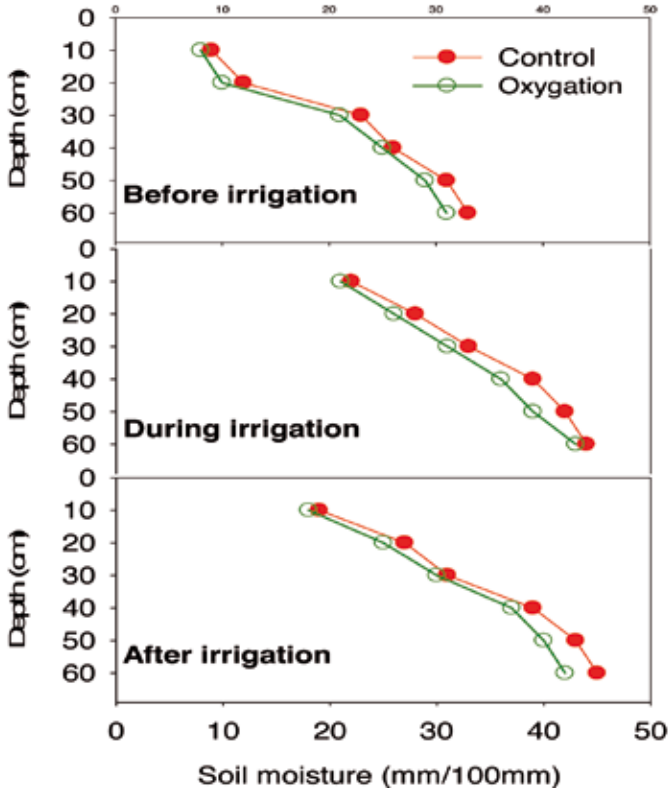


Fig. 5 - Change in soil moisture (mm water 100 mm^{-1} of soil depth) over a period of four days [before (2 days), during (upon completion of 2 hr irrigation) and after (2 days) irrigation] in the oxygation and control irrigation.

(Fig. 6). A higher oxygen concentration in the rhizosphere during oxygation events compared to non-aerated water irrigation was also reported by Chen *et al.* (2010) in cotton and wheat crops in both vertisol and ferrosol under similar climatic conditions.

greater soil resistance in the wetting fronts of cotton under oxygation compared to the control. Such a response was linked with rapid water uptake and transpiration by oxygated plants and, therefore, a drier soil in the wetting front region that contributed to greater soil resistance.

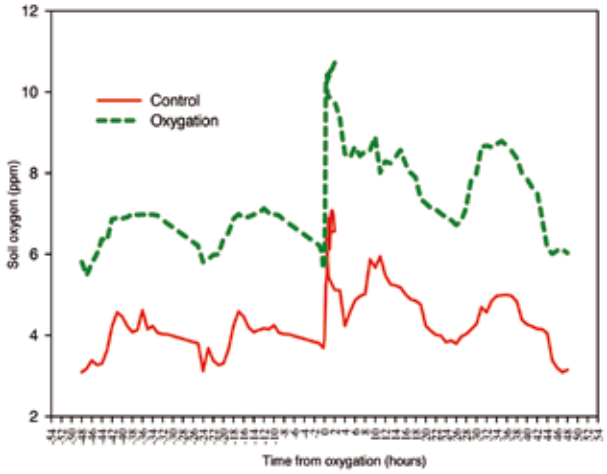


Fig. 6 - Change in soil dissolved oxygen concentration (ppm) over the period of four days [before (2 days), during (upon completion of 2 hr irrigation cycle) and after irrigation (2 days)] in the oxygation and the control treatment measured at the wetting front.

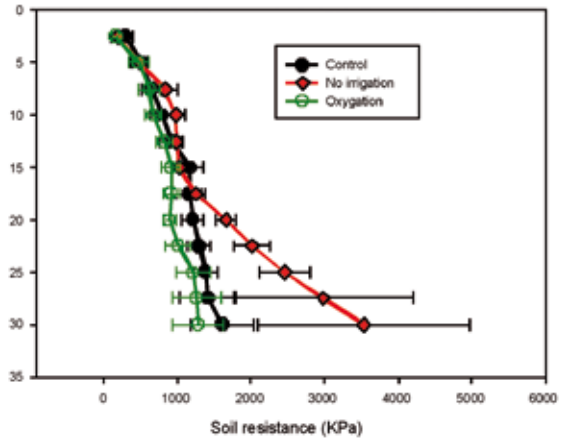


Fig. 7 - Soil compaction measured in the soil at field capacity at the end of the ratoon crop as influenced by soil depth for oxygation (open circle), control (closed circle), and non-irrigated (closed diamond) treatments.

Changes in soil physical properties

Soil compaction. There was less soil compaction in the oxygation treatment compared to the control, and it was higher in the non-irrigated treatment, particularly at depths below the position of emitters (Fig. 5). Soil resistance increased down the soil profile to 37.5 cm depth, and the treatment effect was consistent over this depth. The data suggest that the lower soil resistance down the profile was consistently maintained throughout the profile in the oxygation treatment (Fig. 7) compared to the control and no-irrigation treatments, particularly in the positions below the emitter depths. This observation is in contrast to the report presented by Bhattarai and Midmore (2010 unpublished data) on cotton in vertisol where they showed a

Soil bulk density and air filled porosity. A tendency for lower air filled porosity was recorded in the no-irrigation treatment compared to irrigated oxgation and control treatments. Air-filled porosity at field capacity did not differ significantly between the treatments (Table 1). However, the air field porosity at near saturation was significantly greater in the irrigated control and higher in oxygation compared to the no-irrigation treatment. While no significant difference between the treatments was noted for the soil bulk density, root density was significantly lower (reduced by one half) for the no-irrigation treatment compared to both oxygation and control treatment (Table 1). Non irrigated plants showed shallower root systems, and were easier to pull by hand. Instead of putting down deep rooting, they tended to produce adventitious root in the soil surface. Consistent with these results, Schneider *et al.*

Table 1 - Soil physical and biological properties assessed at the end of the ratoon crop for oxygation, control and no-irrigation treatments

Treatments	Air filled porosity (%)		Bulk density (g/cm^3)	Root density (kg/m^3)	Fluorescence release (µg /g dwt soil/ hr)	*Phytophthora* (% plants)
	Near saturation	Field capacity				
Control	2.6	20.7	1.68	6.32	2.2	4.9
Oxyation	2.3	20.6	1.61	7.50	2.2	3.0
No-irrigation	2.0	18.3	1.61	3.56	1.8	10.5
P value	0.021	0.102	0.243	0.047	0.890	<0.001
LSD (p≤0.05)	0.537	NS	NS	1.811	NS	1.379

Mean separated by LSD.
NS= not significant.

(1992) reported deep penetration of roots and greater root biomass near the drip line and major concentration of root mass at 30-40 cm depth in drip irrigated pineapple on a silty clay soil in Hawaii USA.

Soil respiration

In the ratoon crop, the rate of soil respiration did not differ significantly between the oxygation and control treatments three days after irrigation, however the rate was greater for the irrigated treatments compared to no-irrigation treatment. The diurnal pattern of soil respiration showed a greater soil respiration rate during the day and during the night compared to the early morning and evening (Table 2). In the fast growing pineapple crop, i.e. before the first crop harvest, soil respiration was significantly greater in the oxygation (2.2 g CO_2 m^{-2} hr^{-1}) compared to the control (1.4 g CO_2 m^{-2} hr^{-1}) treatment, an increase of 64%, at 394 days after planting (Chen *et al*., 2010), and following 6 hr of irrigation. Root respiration has been shown to be enhanced by aeration in a number of previous studies on oxygation. These findings are in accord with those of Bhattarai *et al*. (2005) who showed that oxygation increases the amount of oxygen in the irrigation water and ultimately in the root zone, which drives greater root respiration, and therefore ameliorates the temporal hypoxia associated with wetting fronts.

Table 2 - Soil respiration, soil temperature, and soil resistance for different irrigation treatments and diurnal sampling

Treatments	Soil respiration (g CO_2/m^2)	Soil temperature (°C)	Soil resistance (kPa) (2.5-30 cm)
Control	0.82	26.8	1285.2
Oxygation	0.82	26.5	1012.8
No-irrigation	0.37	27.1	2067.5
P value (Aeration)	0.018	0.030	<0.001
LSD (p≤0.05) (Aeration)	0.329	0.423	434.1
Day (1300 hrs)	0.89	29.5	-
Evening (1900 hrs)	0.51	26.2	-
Night (2300 hrs)	0.99	26.1	-
Morning (0500 hrs)	0.57	25.2	-
P value (Diurnal)	0.008	<0.001	
P value (A x D)	0.287	0.726	
LSD (p≤0.05) (Diurnal)	0.327	0.420	-
LSD (A x D)	NS	NS	-

Mean separated by LSD.
NS= not significant.

Soil biological properties and Phytophthora

Low FDA values were noted in the no-irrigation treatment compared to irrigation treatments without significant differences. A marked effect of irrigation treatments was noted on the development of *Phytophthora* symptoms in the field crop (Table 2). The oxygation treatment showed significantly lower infection (3%) compared to the no oxygation SDI treatment (4.9%), whereas the highest *Phytophthora* infestation (10.5%) was recorded in the no-irrigation treatment. In spite of the lower water application rate and reasonably dry soil surface in the no-irrigation plot, development of *Phytophthora* was more severe in this treatment. Exposure of the roots to the soil surface provides poor anchorage to the plant. When the plant was loaded with fruit, the top-heavy weight of the plant resulted in crop lodging and damage to the roots. This may have predisposed the plants to *Phytophthora* contamination particularly when the plot was wet due to rainfall. Severe crop lodging was noted in the non-irrigated treatment in this trial site. A study by Stirling (2004) also suggested that the pineapple crop in QLD in a ferrosol treated with cane trash mulch and under minimum tillage, both of which contribute to maintaining greater soil aeration status, recorded a high FDA level and a positive correlation with greater nematode suppression in the field compared to non-mulched traditional tillage treatments.

Soil chemical properties (nutrients)

Soil organic carbon, Colwell K and Colwell P contents were higher (the latter two not reaching significance at P<0.05) in the no-irrigation treatment compared to oxygation and control treatments when analysed at the end of the crop period. In contrast, soil pH ($CaCl_2$) was significantly lower, and exchangable K was signifcantly higher in the no-irrigation treaments compared to irrigated control or oxygation treatments (Table 3). No significant effects of irrigation treatments were detected for total N, electrical conductivity, and exchangeable calcium and magnesium concentration in the soil.

Table 3 - Macro nutrients, organic carbon, soil pH, conductivity (Cond) and exchangeable calcium (Exc. Ca), exchangeable magnesium (Exc. Mg), exchangeable potassium (Exc. K) in the soil sampled after the ratoon crop in oxygation, control and no-irrigation treatments

Treatments	Organic carbon (%)	N Total (%)	Colwell P (mg/kg)	Colwell K (mg/kg)	Cond (dS/m)	pH ($CaCl_2$)	Exc. Ca (meq/100g)	Exc. Mg (meq/100g)	Exc. K (meq/100g)
Control	0.900	0.06	19.00	61.50	0.04	3.45	0.22	0.06	0.04
Oxygation	0.890	0.07	17.50	56.50	0.05	3.50	0.18	0.05	0.05
No-irrigation	1.210	0.08	45.00	107.00	0.05	3.30	0.31	0.08	0.12
P value	0.064	0.14	0.009	0.086	0.572	0.033	0.547	0.485	0.008
LSD	NS	NS	NS	NS	NS	0.129	NS	NS	0.031

Mean separated by LSD.
NS= not significant.

Plant growth and development

Dry matter partitioning during growth of the main crop

The effect of oxygation was assessed on vegetative and reproductive biomass of the main crop during the early fruit growth stage prior to maturity (392 DAT). Dry weight in the root, leaf, fruits and total above-ground dry biomass increased significantly due to oxygation compared to the control. However, the stem dry weight was not affected by the treatments. The size of the immature fruits (measured as weight of individual fruit) was significantly larger with oxygation compared to the control harvested at the same time (Table 4).

The fruit dry weight was greater by 14% with oxygation compared to the control and, dry biomass was 13% greater in the oxygation compared to the control treatment (Table 4). These results are consistent with yield increases in previous trials on other crops such as tomato, zucchini and cotton representing the C_3 pathway for CO_2 assimilation (Bhattarai *et al*., 2005).

Dry matter partitioning during the growth of the ratoon crop

Dry matter partitioning was carried out for the ratoon crop during the growth phase, at the same time as the diurnal measurements of gas exchange (713 DAT). Above-ground and total dry matter biomass increased with oxygation compared to the control, but without significant differences. However, the increase in leaf dry weight associated with oxygation, compared to the control, was significant (Table 5). The leaf weight with oxygation at 22.9 t/ha was 15% greater than that for the control. This result in the ratoon crop was similar to that in the main pineapple crop, where leaf weight increased by 14%.

The total biomass was 35.4 t/ha with oxygation, which was 7% more than in the irrigated control (31.2 t/ha). This result is also consistent with, but somewhat less than, the result in the main pineapple crop, where the biomass increase was 14%. The oxygation treatment improved root biomass in the main crop (79%) and much less so in the ratoon crop compared to the non-oxygated control. The result in the main crop was consistent with the hypothesis that oxygation improves oxygen availability in the rhizosphere, which positively influences the availability and uptake of water and nutrients favorable for increased root growth and enhanced soil microbial functions (Goorahoo *et al.,* 2002). The lower soil compaction in oxygation treatment plots could also have favoured root growth.

Crop physiological performance

Leaf chlorophyll

Leaf chlorophyll content was estimated using a SPAD meter. A standard calibration for SPAD was also made with acetone chlorophyll extraction method (Arnon, 1954) and a close agreement was achieved between these two methods (Fig. 8) as reflected by the coefficient of determination (R^2=0.807). The chlorophyll content in the D leaf of the ratoon crop was recorded as higher with oxygation compared to the control and no-irrigation treatments. The increase in leaf chlorophyll content was to the order of 11-17% with oxygation compared to that of the control and no-irrigation treatments (Table 6).

Table 4 - Effect of oxygation on plant dry weight and its components at the sample harvest of the main crop (392 DAT) from irrigated control and oxygation treatments (no irrigation treatments were not sample harvested in main crop)

Treatments	Stem (g/m^2)	Root (g/m^2)	Leaf (g/m^2)	AGDB [z] (g/m^2)	Fruit (g/fruit)	Total (g/m^2)	Root/Shoot
Control	585.9	582.3	1760.0	2345.9	833	2928.2	0.248
Oxygation	671.8	1056.1	2232.0	2903.8	1002	3959.9	0.363
P value	0.633	0.004	0.025	0.001	0.016	0.004	0.071
LSD (p≤0.05)	NS	343.5	555.8	323.7	124.7	367.5	0.109

[z] Above-ground dry weight.
Mean separated by LSD.
NS= not significant.

Table 5 - Effect of oxygation on dry plant weight and its components at harvest of the ratoon crop (713 DAT)

Treatments	Stem (g/m^2)	Root (g/m^2)	Leaf (g/m^2)	Crown (g/m^2)	Fruit (g/m^2)	AGDB (g/m^2)	Total (g/m^2)	Root/Shoot
Control	549	528	1942	9.9	85	2587	3115	0.204
Oxygation	598	533	2289	14.7	105	3007	3541	0.177
No-irrigation	623	409	2136	0.0	0.0	2760	3169	0.148
P value	0.76	0.32	0.08	0.62	0.53	0.20	0.22	0.34
LSD (p≤0.05)	NS	NS	150	NS	NS	NS	NS	NS

Mean separated by LSD.
NS= not significant.

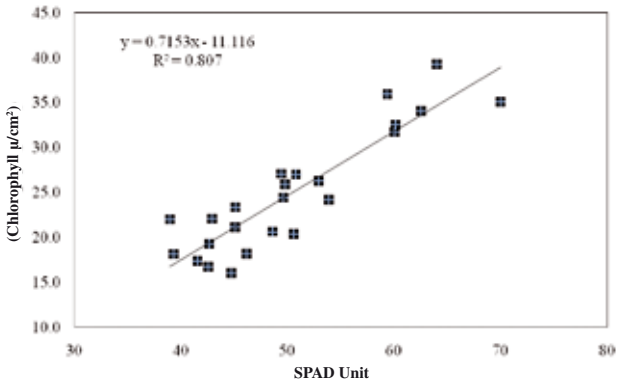


Fig. 8 - Relationship between the SPAD unit and leaf chlorophyll concentration by acetone extraction method.

'D' Leaf characters

A significant increase in plant height was recorded in oxygation and control treatments compared to no-irrigation plants. Although in the first crop there was no effect of oxygation on the number of leaves per plant, nor on 'D' leaf area and the specific leaf area (SLA) of the 'D'leaf (nor on plant height, data not presented), a significant increase in 'D' leaf area due to SDI with or without oxygation compared to the non-irrigated control was evident in the ratoon crop (Table 6). A larger 'D' leaf area has been linked with higher yield of pineapple fruits in a number of previous studies (e.g., Fournier *et al*., 2007).

Canopy light interception

Light interception by the canopy increased significantly with oxygation compared to the control and no-irrigation treatments (Table 6). Light interception by the canopy was highest (92%) in the oxygation treatment and increased by 5% and 7% compared to the no-irrigation and control treatment respectively (Table 6).

Leaf photosynthesis

There was a distinct diurnal pattern for leaf gas exchange. No gas exchange activity was recorded during the daytime, while the carbon dioxide exchange rate ranged between 2.4-3.5 µmol m^2 s^{-1} over the early morning, evening and night (Table 7). A higher CO_2 exchange rate was noted in the oxygation treatment compared to the control

Table 6 - Pineapple leaf characteristics of the ratoon crop as affected by irrigation treatments when harvested at the fruit developing stage

Treatments	Plant height (cm)	Leaf (No./plant)	'D'- Leaf area (cm^2)	'D'- Leaf weight (g)	SLA[z] (cm^2/g)	Chlorophyll (SPAD units)	LI [y] (%)
Control	98.0	9.6	263.23	5.81	46.8	44	87
Oxygation	102.0	8.9	280.45	6.15	48.2	49	92
No-irrigation	93.3	7.9	215.31	5.03	45.4	42	83
P value	0.06	0.82	0.01	0.31	0.63	0.08	0.09
LSD (p≤0.05)	5.8	NS	33.11	NS	NS	5.2	5.6

Mean separated by LSD.
NS= not significant.
[z] SLA = Specific leaf area.
[y] LI = Light interception by the canopy.

Table 7 - Diurnal variation for leaf CO_2 exchange rate, stomatal conductance, transpiration rate, and leaf temperature for different treatments

Treatments	Leaf CO_2 exchange rate (µmol/m^2/s)	Stomatal conductance (mmol/m^2/s)	Transpiration rate (mmol/m^2/s)	Leaf temperature (°C)
Control	2.038	0.312	2.75	30.7
Oxygation	2.198	0.376	2.80	30.6
No-irrigation	1.850	0.150	2.71	31.1
P value (Aeration)	0.39	0.428	0.062	0.033
LSD (p≤0.05)	NS	NS	0.09	0.396
Day (1300 hrs)	-0.14	0.018	1.27	38.5
Evening (1900 hrs)	3.53	0.196	3.21	27.7
Night (2300 hrs)	2.39	0.353	3.69	27.6
Morning (0500 hrs)	2.93	1.324	5.30	27.5
P value (Diurnal)	<0.001	<0.001	<0.001	<0.001
LSD (p≤0.05) (Diurnal)	0.585	0.463	1.034	0.451
P value (A x D)	0.791	0.135	0.082	<0.001
LSD (p≤0.05) (A x D)	NS	NS	NS	0.966

Mean separated by LSD.
NS= not significant.
A x D = Interactions between aeration and diurnal measurement for the given parameters.

and no-irrigation. The diurnal pattern of leaf gas exchange in photosynthesis in pineapple is characteristic of the Crassulacean acid metabolism (CAM), in which carbon dioxide is temporarily fixed during the night and in conditions of very low light intensity as in other succulent plants (Cushman, 2001).

Pineapple crops are able to cope with seasonal variations in weather such as rainfall, dry atmosphere and drought, all of which reduce productivity, due to their ability to assimilate CO_2 via the CAM pathway (San-José *et al.*, 2007). During the day stomata are closed and leaf surface transpiration is at its lowest (Zhu *et al.*, 1999). Due to this unique CAM physiology, pineapple exhibits high WUE, several times higher than C_3 and C_4 plants (Cushman, 2001). Our leaf gas exchange data were collected as point source data, from small portions of the leaf and were instantaneous measurements, hence raising questions as to whether the observations made in a single leaf in instantaneous time frames can reflect the response of the whole plant over an integrated time scale.

Leaf transpiration, stomatal conductance and temperature

Leaf transpiration was low during the day and higher in early morning, evening and night (Fig. 9), and was lower for control and no-irrigation treatments compared to oxygation (Table 7). The stomatal conductance was somewhat higher for the oxygation compared to the control and no-irrigation treatments; however, the difference in stomatal conductance between the treatments was not statistically significant (Fig. 9).

In contrast, the leaf temperature measured during gas exchange decreased significantly with irrigation compared to the no-irrigation treatment (Table 7). Higher leaf transpiration rate is associated with evaporative cooling of the leaf surface that reduces leaf temperature in relation to the ambient temperature of the leaf environment.

A significant interaction between irrigation method and diurnal time scale was due to significantly higher predawn leaf temperature in no-irrigation treatment compared to aerated SDI and SDI control (Table 7). This is due to low transpiration, and slow evaporative cooling of leaf in this treatment compared to other irrigated treatments in the experiment (Table 7).

Fruit yield

The harvest from sub-sample areas at maturity was comprised of all fruits irrespective of their size and marketability. The total pineapple fruit yield (consisting of main crop and ratoon) was significantly greater with oxygation (133.7 t/ha) compared to that of the irrigation control (106.4 t/ha), and least in no-irrigation treatment (90.4 t/ha). The total yield increase due to oxygation was 48% compared to no-irrigation, and 26% compared to the control (Table 8). The harvest yield was greater in the main crop compared to the ratoon crop. The total harvest of the ratoon crop was only 51% that of the main crop, averaged over all three treatments. However, oxygation still maintained a higher yield compared to the control and no irrigation in the ratoon crop.

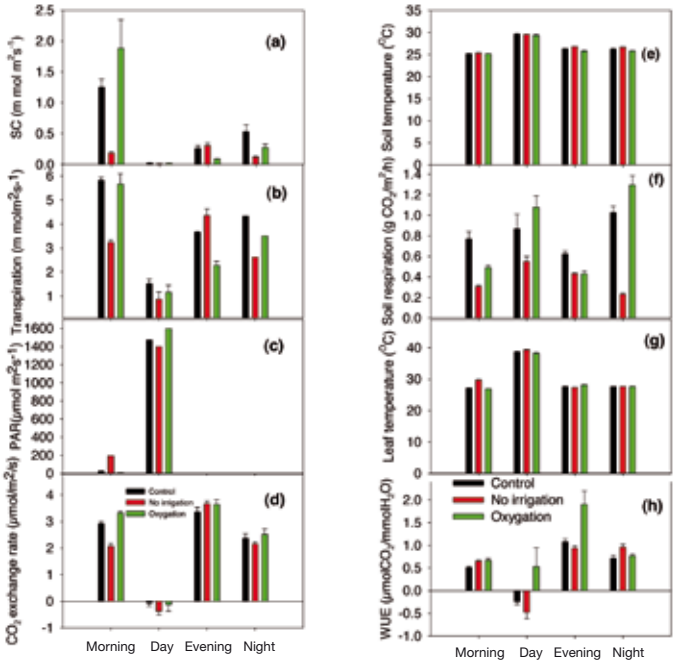


Fig. 9 - Leaf stomatal conductance (a), transpiration rate (b), photosynthetically active radiation (c), CO_2 exchange rate (d), soil temperature (e), soil respiration (f), leaf temperature (g) and instantaneous water use efficiency (h) in oxygation, control and no-irrigation treatment over the time period of 24 hr (bar shows mean, line shows standard error of the means) in a ratoon pineapple crop.

Table 8 - Pineapple fruit yield from the sample area (harvested yield) and industry marketable harvest (industry yield) for the main and ratoon crops

Treatments	Harvested yield (t/ha) [z]			Industry yield (t/ha) [y]		
	Main crop	Ratoon	Total	Main crop	Ratoon	Total
Control	68.20	38.17	106.37	50.92	18.25	69.17
Oxygation	79.60	54.11	133.71	53.08	20.18	73.26
No-irrigation	71.30	19.07	90.37	49.50	16.42	65.92
P value	0.005	0.001	0.032	0.295	0.051	0.076
LSD (p≤0.05)	6.43	10.76	12.36	NS	3.17	7.39

Mean separated by LSD.
NS= not significant.
[z] Harvested yield is all fruits harvested from the sample area.
[y] Industry yield refers to only marketable yield harvested on whole plot basis by the industry picking process.

Total industry fruit yield was highest in the oxygation treatment (73.3 t/ha), followed by control (69.2 t/ha) and no-irrigation (65.9 t/ha). The marketable fruit yield in the industry harvest was greater by 11% due to oxygation compared to the no-irrigation treatment, and 6% compared to the control treatment (Table 8). The industry yield as a proportion of the sampled yield was also greater in the main crop compared to the ratoon crop. The total industry harvest in the ratoon crop was only 36% that of the main crop, averaged over all three treatments. The sample plot yields were considerably higher than the commercially harvested yields due to the fact that commercial yield only considered fruit >1.5 kg; the sample plot yields included fruits which were smaller but mature. The benefits of oxygation for pineapple yields are in agreement with data from oxygation field trials on a vertisol, where lint yield of cotton increased consistently over a number of years and the benefit averaged >10% per annum (Bhattarai and Midmore, 2009).

Fruit quality

The individual fruit weight increased significantly due to oxygation, particularly for the main crop, compared to the control and no-irrigation treatment. The fruits in the oxygation treatment were 230 g and 228 g larger than the control and no-irrigation treatments respectively in the main season crop. In the ratoon crop, the effect of treatments on mean fruit size was not as notable, and the mean fruit size in the no-irrigation treatment had improved considerably. Greater annual rainfall (~1900 mm) that was more evenly distributed compared to the previous year (Fig. 1) minimized crop water stress, and imparted a positive effect on the fruit size and quality in the no-irrigation treatment in the ratoon crop. Other parameters of the fruit size such as fruit height and width were also significantly greater with oxygation compared to the control in the main crop. Such increase in fruit size due to oxygation has also been reported for other crops such as tomato (Bhattarai *et al.,* 2006).

The total soluble solid content measured as °brix and dry matter content remained quite consistent across seasons and treatments. The brix readings were much higher than the minimum brix standard set for Golden Circle (12° brix) and fresh market consumption.

A number of other fruit quality parameters such as fruit translucency, flesh and skin color, flavor and fruit shape were also measured at harvest. Fruit translucency at harvest in the main crop was lower with oxygation compared to that of the control treatment (Table 9), whereas in the ratoon crop the effect of oxygation was significant in lowering the translucency. Low translucency in pineapple at harvest is considered an indicator of better quality fruit. Fruit quality measured by ranking of flesh colour was better under the oxygation: the score for flesh colour ranking was 11% higher (3.29) under oxygation than under the control (2.95). Fruit quality measured by ranking of skin colour was also better in the oxygation treatment. The score for skin colour was 5% higher with oxygation than in the control. Flavor quality of pineapple was also improved with oxygation. The flavor quality score for the sample from the oxygation treatment was 12% higher than the control sample. Although a positive effect of oxygation was recorded on these quality parameters, the differences were not statistically significant in either crop, except for flavor in the main crop and translucency in the ratoon (Table 10).

Water use efficiency

Season-long water use efficiency

For the total harvested yield component, the irrigation water use efficiency (IWUE), which includes only the irrigation component as the water input, increased by 20% due to oxygation (52.98 t/ML) compared to the control (44.23 t/ML). The gross water use efficiency (GWUE), which includes both irrigation and rainfall inputs, increased by 39% due to oxygation (2.97 t/ML), and by

Table 9 - Fruit characteristics and quality parameters of pineapple as affected by irrigation treatments in the main crop

Treatments	Fruit weight (g/fruit)	Fruit height (cm)	Fruit width (cm)	Brix (º)	Density (g/cm^3)	Dry matter (%)	Translucency (1-5) [z]	Flavor (1-3) [y]	Flesh colour (1-5) [x]	Skin colour (1-5) [w]
Control	832.0	12.71	10.32	16.41	0.89	17.51	1.28	2.45	2.95	2.93
Oxygation	1061.8	13.77	10.85	16.55	0.91	17.76	1.10	2.75	3.29	3.07
No-irrigation	834.0	14.50	10.63	15.83	0.97	17.57	2.17	2.83	3.67	3.50
P value	0.045	0.014	0.084	0.719	0.185	0.547	0.11	0.005	0.197	0.564
LSD (p≤0.05)	209.5	0.748	NS	NS	NS	NS	NS	0.167	NS	NS

Mean separated by LSD.

NS= not significant.

[z] Translucency rating: 1 = 0% translucency, 2 = 25% translucency, 3 = 50% translucency, 4 = 75% translucency, 5 = 100% translucency.

[y] Flavor rating: 1 = No flavor, 2 = little flavor, 3 = good flavor.

[x] Flesh colour rating: 1 = 100% white, 2 = 25% yellow, 3 = 50% yellow, 4 = 75% yellow, 5 = 100% yellow.

[w] Skin colour rating: 1 = 100% green, 2 = 25% yellow, 3 = 50% yellow, 4 = 75% yellow, 5 = 100% yellow.

Table 10 - Fruit characteristics and quality parameters of pineapple as affected by irrigation treatments in the ratoon crop

Treatments	Fruit weight (g/fruit)	Fruit height (cm)	Fruit width (cm)	Brix (º)	Density (g/cm^3)	Dry matter (%)	Translucency (1-5) [z]	Flavor (1-3) [y]	Flesh colour (1-5) [x]	Skin colour (1-5) [w]
Control	793.0	12.40	10.17	16.46	0.90	16.37	1.30	2.41	2.74	2.76
Oxygation	961.0	13.43	10.62	13.80	0.92	16.61	0.92	2.29	2.74	3.22
No-irrigation	988.0	14.68	10.70	15.90	0.97	16.81	2.40	2.80	3.80	3.60
P value	0.061	0.007	0.138	0.09	0.003	0.674	0.003	0.117	0.077	0.473
LSD (p≤0.05)	203.0	1.16	NS	NS	0.03	NS	0.638	NS	NS	NS

Mean separated by LSD.
NS= not significant.
[z] Translucency rating: 1 = 0% translucency, 2 = 25% translucency, 3 = 50% translucency, 4 = 75% translucency, 5 = 100% translucency.
[y] Flavor rating: 1 = No flavor, 2 = little flavor, 3 = good flavor.
[x] Flesh colour rating: 1 = 100% white, 2 = 25% yellow, 3 = 50% yellow, 4 = 75% yellow, 5 = 100% yellow.
[w] Skin colour rating: 1 = 100% green, 2 = 25% yellow, 3 = 50% yellow, 4 = 75% yellow, 5 = 100% yellow.

9% due to the control (2.37 t/ML) compared to the no-irrigation treatment (2.20 t/ML) (Table 11).

For the marketable yield component (i.e. the industry harvest), IWUE increased only marginally due to oxygation, while GWUE increased by 6 % due to oxygation (1.63 t/ML), and by 5% due to the control (1.54 t/ML) compared to the no-irrigation treatment (1.55 t/ML) (Table 11).

Table 11 - Water use efficiency (irrigation water use efficiency and total water use efficiency) of the harvested sample yield and industry yield in different irrigation treatments for the total yield averaged over the main and ratoon crop

Treatments	WUE harvested yield (tonnes/ML)		WUE industry yield (tonnes/ML)	
	IWUE [z]	GWUE [y]	GWUE	GWUE
Control	44.23	2.37	28.76	1.54
Oxygation	52.98	2.97	29.02	1.63
No-irrigation	NA	2.13	NA	1.55
Mean	48.60	2.49	28.89	1.57

[z] IWUE= Irrigation water use efficiency presents tonnes of total harvested fruits per mega liter of applied irrigation.
[y] GWUE= Gross water use efficiency presents tonnes of total harvested fruits per mega liter of applied irrigation + rain contribution in the crop for the entire crop duration. The WUE has been presented for harvested yield (total harvest from the sample area), and industry yield harvested by the industry harvesting crew by the machine (represent total marketable fruits).

These observations are consistent with, but much smaller than, the findings of Bhattarai *et al*. (2005) where greater WUE due to oxygation using SDI tomato was reported, and for cotton and vegetable soybean where season-long WUE for fruit and biomass yield and instantaneous leaf transpiration rate were greater with oxygation (Bhattarai and Midmare, 2009).

Cost benefit analysis/decision support

The additional cost for installing an oxygation unit in an already established sub-surface drip irrigation system involves the purchase of a Mazzei air injector model MI 1583 (AU$ 365), plus fittings and pressure gauges for an existing 3" irrigation pipe (AU$ 135), totaling AU$ 500/ha. The installation cost per unit area can decrease with an increase in the size of the air injector. The estimated yield increment of 7.5 ton/ha/crop with oxygation over the average industry yield of 65.9 ton/ha without irrigation brings an additional return of AU$ 3750/ha in the first crop at a sale value of AU$ 500/ton of fruits for the investment of AU$ 500 for oxygation.

For a new SDI installation, however, the cost with oxygation for pineapple is AU$ 6000. Hence, the repayment period for the investment to oxygated SDI is two crop cycles (six years). SDI infrastructure lasts 15 years, covering five cycles of the crop (three years/crop cycle) with potential additional returns of $18,750/ha over the 15-year period. These comparative estimates have been based on a crop with no-irrigation, particularly in high rainfall years (4500 mm over three years). Crop performance under drier years without irrigation is expected to be much less and under such circumstances SDI offers greater opportunity to deliver strategic irrigation. We conclude that oxygation can improve both yield and quality of ratoon pineapple for an industry scale of operation.

4. Conclusions

The total and marketable fruit yield increased with oxygation and the irrigation control compared to no-irrigation. Aerated water increased the marketable fruit yield (73.25 t/ha) by 11% whereas control treatment increased yield (69.2 t/ha) by 6% compared to no-irrigation (65.9 t/ha). Total yield (both marketable and unmarketable) was greater, significantly so, due to oxygation (133.7 t/ha), compared to the control (106.4 t/ha), and no-irrigation (90.4 t/ha). Yield gain with oxygation was attributed to the larger area and weight per leaf, greater plant height, higher specific leaf area, chlorophyll content and light interception by the canopy compared to the control and no-irrigation. Greater CO_2 exchange rates and instantaneous water use efficiency were recorded for the oxygation compared to

the control and no-irrigation treatments. Carbon dioxide exchange was not measurable during the day, only in the early morning, evening and night.

Aerated irrigation water also reduced *Phytophthora* infestation in the field from 11% in the non-irrigated control to 3%, whereas 5% infestation was noted for control drip irrigation. Hence, the use of aerated drip irrigation demonstrated multiple benefits for yield, quality and *Phytophthora* disease management. The trial seasons were rather wetter than average years, and the in crop total rainfall was 4250 mm (42.5 ML), requiring only small amounts of irrigation (2.405 and 2.524 ML for control and oxygation respectively). Supplementary and strategic irrigation contributed only 5.5% to total crop water input. Nevertheless, this strategic use of irrigation and oxygation led to marked benefits for the pineapple crop yield and quality.

Acknowledgements

This research was supported by Horticulture Australia Limited (HAL) and the National Program for Sustainable Irrigation (NPSI) Australia. We thank Valley Syndicate farm for their active participation and support.

References

ADAM G., DUNCAN H., 2001 - *Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils.* - Soil Biol. Biochem., 33: 943-951.

ADAMS W.W., ADAMS B.D., ROSENSTIEL T.N., 2002 - *Photosynthesis and photoprotection in over-wintering plants*. - Plant Biol., 4: 545-557.

ARNON D.I., 1954 - *The role of micronutrients in plant nutrition with special reference to photosynthesis and nitrogen assimilation*, pp. 1-32. - In: LAMB C.A., O.G. BENTLEY, and J.M. BEATTIE (eds.) *Trace elements*. Academic Press, New York, USA.

BARTHOLOMEW D.P., 2008 - *Estimating plant weights*. - Pineapple News, 15: 2-5.

BARTOLOME A.P., RUPEREZ P., FUSTER C., 1995 - *Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne cultivars*. - Food Chem., 53: 75-97.

BHATTARAI S., HUBER S., MIDMORE D.J., 2004 - *Aerating subsurface irrigation gives growth and yield benefits to zucchini, vegetable soybean and crops in heavy clay soils*. - Ann. Appl. Biol., 144: 285-298.

BHATTARAI S., SU N., MIDMORE D.J., 2005 - *Oxygation unlocks yield potentials of crops in oxygen limited soil environments*. - Adv. in Agron., 88: 313-377.

BHATTARAI S.P., MIDMORE D.J., 2009 - *Oxygation enhances growth, gas exchange and salt tolerance of vegetable soybean and cotton in saline vertisol*. - J. Integ. Pl. Biol., 51: 675-688.

BHATTARAI S.P., PENDERGAST L., MIDMORE D.J., 2006 - *Oxygation of subsurface drip irrigated tomato (*Lycopersicon esculentum L.*) improves yield performance, tolerance to salinity and water use efficiency in normal and saline heavy clay soil*. - Sci. Hortic., 108: 278-288.

CAMP C.R., GARRETT J.T., SADLER E.J., BUSSCHER W.J., 1993 - *Microirrigation management for double-cropped vegetables in a humid area*. - Trans. ASAE., 36(6): 1639-1644.

CHEN X.M., DHUNGEL J., BHATTARAI S.P., TORABI M., PENDERGAST L., MIDMORE D.J., 2010 - *Impact of oxygation on soil respiration, yield and water use efficiency of three crop species*. - J. Plant Ecol., DOI: 10.1093/jpe/RTQ030.

COLLINS J.L., 1960 - *The pineapple: Botany, cultivation and utilisation*. - Interscience Publishers, New York, USA.

CUSHMAN J.C., 2001 - *Cassulacean acid metabolism. A plastic photosynthetic adaptation to arid environments*. - Plant Physiol., 127: 1439-1448.

DHUNGEL J., MIDMORE D.J., WALSH K.B., CHEN X.M., BHATTARAI S.P., SUBEDI P.P., 2009 - *Oxygation enhanced pineapple yield and quality*. - Acta Horticulturae, 889: 551-556.

DOGAN E., KIRNAK H., BEREKATOGLU K., BILGEL L., SURUCU A., 2008 - *Water stress imposed on muskmelon (*Cucumis melo *L.) with subsurface and surface drip irrigation systems under semi-arid climatic conditions*. - Irrigation Sci., 26: 131-138.

FAO, 2011 - *Pineapple area, production and yield in the world for 2009*. - FAOSTAT© FAO Statistics Division 2011. - http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.

FOURNIER P., SOLER A., MARIE-ALPHONSINE P.A., 2007 - *Growth characteristics of the pineapple cultivars 'MD2' and 'Flhoran 41' compared with 'Smooth Cayenne'*. - Pineapple News, 14: 18-20.

GOORAHOO D., CARSTENSEN G., ZOLDOSKE D.F., NORUM E., MAZZEI A., 2002 - *Using air in sub-surface drip irrigation (SDI) to increase yields in bell peppers.* - International Water Irrigation, 22(2): 39-42.

HANSON P.J., EDWARDS N.T., GARTEN C.T., 2000 - *Separating root and soil microbial contributions to soil respiration: a review of methods and observations*. - Biogeochemistry, 48: 115-146.

KLIMANT L., MEYER V., KUHL M., 1995 - *Fibre-optic oxygen micro-sensors, a new tool in aquatic biology*. - Limnol Oceanogr., 40: 1159-1165.

KUHLBUSCH T.A.J., 1995 - *Method for determining black carbon in residues of vegetation fires.* - Env. Sci. & Tech., 29(10): 2695-2702.

MACHADO R.M.A., OLIVEIRA M., DO ROSARIO G., PORTAS C.A.M., 2003 - *Tomato root distribution, yield and fruit quality under subsurface drip irrigation*. - Plant and Soil, 255: 333-341.

MIDMORE D.J., BHATTARAI S.P., PENDERGAST L., SU N., 2006 - *Application of multigation to horticultural crops*. - International Society for Horticultural Science, 27th International Horticultural Congress and Exhibition, Seoul, Korea, 13-19 August.

PEGG K.G., 1977 - *Soil application of elemental sulphur as a control of* Phytophthora cinnamomi *root and heart rot of pineapple*. - Australian Journal of Experimental Agriculture and Husbandry, 17: 859-865.

PEVERILL K.I., SPARROW L.A., REUTER D.J., 2002 - *Soil Analysis: an Interpretation Manual.* - CSIRO publication Australia, pp. 365.

SAN-JOSÉ J., MONTES R., NIKONOVA N., 2007 - *Diurnal patterns of carbon dioxide, water vapour, and energy fluxes in pineapple* [Ananas comosus *(L.) Merr. cv. Red Spanish] field using eddy covariance*. - Photosynthetica, 45(3): 370-384.

SCHNEIDER R.C., ZHANG J., ANDERS M.M., BARTHOLOMEW D.P., CASWELL-CHEN E.P., 1992 - *Nematicide efficacy, root growth, and fruit yield in drip-irrigated pineapple parasitized by* Rotylenchulus reniformis. - Journal of Nematology 24(4): 540-547.

STIRLING G., 2004 - *Manipulating the soil biology to suppress nematode pests.* - Pineapple News, 11: 4-8.

SU N., MIDMORE D.J., 2006 - *Two-phase flow of water and air during aerated subsurface drip irrigation*. - J. Hydrology., 313: 158-165.

THOMPSON T.L., DOERGE T.A., GODIN R.E., 2002 - *Subsurface drip irrigation and fertigation of broccoli: I. Yield, quality, and nitrogen uptake*. - Soil Sci. Soc. Am. J., 66: 186-192.

ZHU J., GOLDSTEIN G., BARTHOLOMEW D.P., 1999 - *Gas exchange and carbon isotope composition of* Ananas comosus *in response to elevate CO_2 and temperature*. - Plant Cell & Environment, 22(8): 999-1007.

Adv. Hort. Sci., 2012 26(1): 17-20

Effects of trinexapac-ethyl on stolon development in potted Patriot bermudagrass

A. Lenzi, A. Baldi, M. Nannicini, A. Pardini, R. Tesi
Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Università degli Studi di Firenze, Piazzale delle Cascine, 18, 50144 Firenze, Italy.

Key words: internode length, nursery production, plant growth regulators, sprigs, warm season turfgrasses.

Abstract: A recent technique developed for establishment of warm season turfgrasses is based on the transplant of single plug plantlets pre-rooted in the nursery. Plantlets are obtained from one-node sprigs about 2 cm long derived from stolon fragmentation. Usually, stolons must be cut several times to obtain sprigs of the right length because of overly long internodes. In the present study, potted plants of Patriot bermudagrass grown in the nursery were treated with trinexapac-ethyl (TE) at the rates 0.1, 0.2, 0.4 and 0.8 kg a.i. ha^{-1}. TE application was aimed at obtaining internode shortening in order to facilitate the stolon division practice. In fact, TE-treated plants showed a decrease in the average length of internodes with respect to control at any applied rate. Nevertheless, the lowest rate applied (0.1 kg a.i. ha^{-1}) did not assure a prolonged effect while the highest rate (0.8 kg a.i. ha^{-1}) caused a decrease in the yield of sprigs. Therefore, our results suggest that TE may be advantageously used and at rates of 0.2-0.4 kg a.i. ha^{-1} to control stolon development of Patriot bermudagrass for nursery purposes.

1. Introduction

Although in Italy lawns are traditionally established with cool season grasses, the use of warm season species has been increasing over the last two decades (Croce *et al*., 2004; Volterrani *et al*., 2010). Warm season grasses show several advantages over cool season species. They tolerate high temperatures and drought, show lower water consumption, tolerate higher salinity concentrations in the soil and in irrigation water, establish rapidly and present excellent recovery properties due to the abundant production of stolons and rhizomes (Biran *et al*., 1981; Carrow and Duncan, 1988; Beard, 1989; Dudeck and Peacock, 1993; Beard and Sifers, 1997; Volterrani *et al*., 1997; Croce *et al*., 2006; Harivandi and Marcum, 2008).

Bermudagrasses (*Cynodon* spp.) are the warm season turfgrass species most widely used in the transition and warm regions of the world due to their aggressive growth habit, tolerance to a wide range of mowing heights, marked resistance to many abiotic stresses, and the high quality of the cultivars recently developed by breeding programs (Wu *et al*., 2009).

A technique recently developed for turf establishment of warm season species is based on the transplant of single plug plantlets pre-rooted in the nursery (Volterrani *et al*., 2008). Nursery activity consists of the pot cultivation of donor plants from which stolons are collected, divided into one-node sprigs about 2 cm long, then cultivated in alveolate trays until the formation of mature plantlets. The crucial point of this practice is stolon fragmentation. This operation is very time consuming and consequently labour-costly. In fact, stolons must be cut several times to obtain sprigs of the right length due to long internodes.

The effectiveness of trinexapac-ethyl (TE) in controlling bermudagrass growth is reported by many authors (Johnson, 1994, 1997; Fagerness and Yelverton, 1999; Lowe and Whitwell, 1999; Fagerness and Yelverton, 2000; Fagerness *et al*., 2002; Bunnel *et al*., 2005; McCullough *et al*., 2005; Baldwin *et al*., 2006; McCullough *et al*., 2007; Baldi *et al*., 2010). In these reports, TE is applied to bermudagrass turfs in order to facilitate their management (mainly by reducing mowing requirements), ameliorate their quality or enhance stress tolerance (e.g. salinity tolerance or cold tolerance). The rates of TE normally adopted in bermudagrass turf management are around 0.11 kg a.i. ha^{-1}, which is the recommended rate for bermudagrass maintained at golf fairway height (Cooper, 2003). Plant height, texture, density, clipping weight and root mass are the growth parameters related to plant response to TE to be considered.

On the contrary, since turf establishment by means of pre-rooted plantlets propagated by stolon in the nursery has been recently developed, the effects of plant growth regulators (PGRs) on grass plants grown to produce propagating material have not been investigated. Furthermore, parameters such as stolon length, internode length and stolon number, important for nursery activity, have been seldom considered.

Traditionally, PGRs are classified into two main groups, Type I and Type II, according to their biological mode of

Received for publication 20 September 2011
Accepted for publication 24 January 2012

action. Type I PGRs inhibit cell division and differentiation in the plant meristems and are excellent seedhead inhibitors; Type II compounds suppress growth by inhibiting the biosynthesis of gibberellic acid, which is needed for cell elongation (Watschke *et al*., 1992).

In turfgrass, three of the most commonly used PGRs [flurprimidol (FL), trinexapac-ethyl (TE) and paclobutrazol (PBZ)] are Type II chemicals (Lowe and Whitwell, 1999). Turf plants treated with Type II PGRs usually show an altered morphology of leaves and a more compact growth habit due to a reduced internode length (Rossi, 1993). Therefore, for their mode of action, Type II PGRs may presumably be useful to reduce stolon length by controlling internode elongation.

In this paper, the effects of TE on potted plants of bermudagrass grown in the nursery are described. The aim of the TE treatment was to obtain a stolon shortening to facilitate the stolon division practice and, hopefully, without reducing the overall production in sprigs, that result from the number of stolons per plant and the number of nodes per stolon.

2. Materials and Methods

Plant material, growing conditions and experimental treatments

The experiment was carried out between April and June 2010 at the Pacini Horticultural Nursery located in Rigoli (Pisa), central Italy (45°45' N, 10°26' E, 6 m a.s.l.).

On 7 April 2010, plantlets derived from one-node sprigs of bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] cv. Patriot were transplanted in 20 cm-diameter, 3.80 l volume polyethylene pots (three plantlets per pot) filled with peat (Baltikum-S132; 92% organic matter, pH 5.5-6.5, electrical conductivity 0.6-1.0 dS m^{-1}) fertilized with 2 g l^{-1} of Osmocote-Pro (Scotts) 18-10-11 + 2 MgO.

On 12 May 2010, after stolons had started to develop, plants were trimmed down to the pot rim height and five treatment rates [0 (= control), 0.1, 0.2, 0.4 and 0.8 kg a.i. ha^{-1}] of TE [(4-cyclopropyl-α-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid methyl ester] were applied by foliar spraying (1250 l ha^{-1}) of the commercial product Primo Maxx (Syngenta).

During the experiment, plants were irrigated as needed by an overhead sprinkler system. During the period, average maximum and minimum temperatures were 35°C and 20°C, respectively, while relative humidity (R.H.) reached 55% and 70% as minimum and maximum values, respectively.

A randomized block design was adopted with five replicates per treatment (1 replicate = 1 pot).

Data collection

After treatment, stolon length and the number of nodes per stolon were recorded weekly for seven weeks on three stolons per pot, and average internode length was calculated. On 30 June 2010, seven weeks after treatment, fresh weight and dry weight of aerial biomass (after oven-drying at 80°C for 48 hr) and the number of stolons of each pot were determined. In addition, the total number of sprigs per pot was calculated as the product of the number of stolons per pot and the final number of nodes per stolon.

Statistics

Data were subjected to one-way analysis of variance (ANOVA) and means were compared using the LSD test at P≤0.05 level of significance.

3. Results

TE induced a statistically significant shortening of stolons compared to the control one and two weeks after application at the rates 0.2, 0.4 and 0.8 kg a.i. ha^{-1} (Fig. 1). Three weeks after treatment, the effect of TE treatments on stolon length began to wane (Fig. 1).

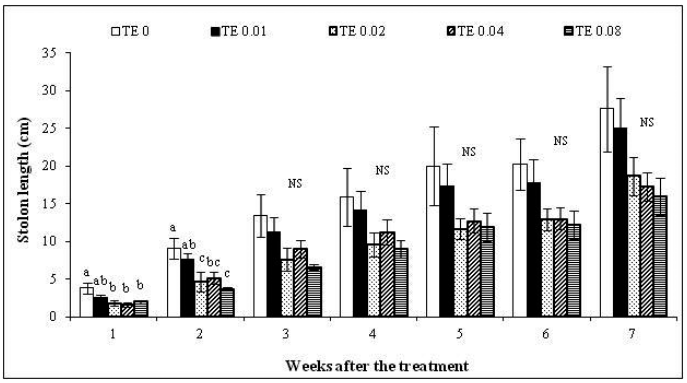


Fig. 1 - Stolon length in Patriot bermudagrass treated with different rates of TE (kg a.i. ha^{-1}). Within the same week, different letters show significantly different values for P≤0.05 (LSD test). Vertical bars show SE of the means (n=5); NS= not significant.

During the entire monitoring period (seven weeks), no statistically significant differences were observed between TE-treated plants and control plants in the number of nodes per stolon (Fig. 2).

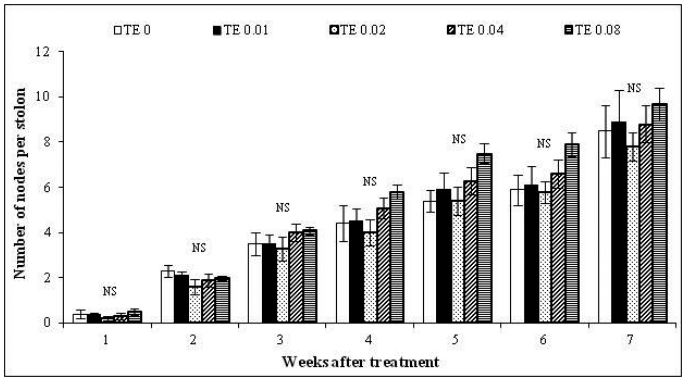


Fig. 2 - Number of nodes per stolon in Patriot bermudagrass treated with different rates of TE (kg a.i. ha^{-1}). Within the same week, different letters show significantly different values for P≤0.05 (LSD test). Vertical bars show SE of the means (n=5); NS= not significant.

TE-treated plants showed a decrease in the average length of internodes (Fig. 3). In control plants internode length varied between 2.7 and 3.0 cm; in TE-treated plants stolons showed an average internode length varying from 1.8 (first week) to 2.5 cm (seventh week) at the rate 0.1 kg a.i. ha^{-1}, from 1.4 (first week) to 2.1 cm (seventh week) at the rate 0.2 kg a.i. ha^{-1}, from 1.2 (first week) to 1.8 cm (seventh week) at 0.4 kg a.i. ha^{-1}, and from 1.2 (third week) to 1.5 cm (seventh week) at 0.8 kg a.i. ha^{-1} (Fig. 3). The differences between control and TE-plants were statistically significant at all the applied rates one week after treatment and afterwards at the rates 0.2, 0.4 and 0.8 kg a.i. ha^{-1}.

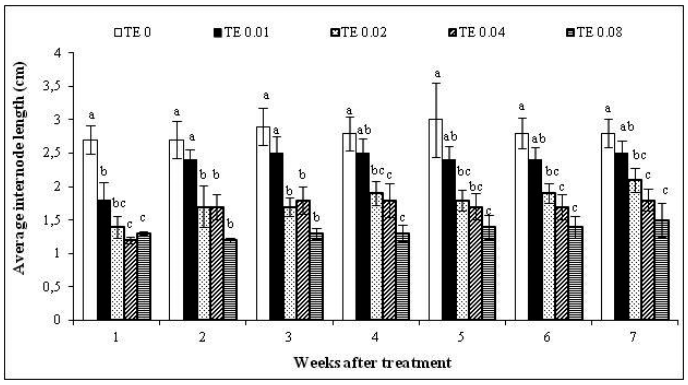


Fig. 3 - Average internode length in Patriot bermudagrass treated with different rates of TE (kg a.i. ha^{-1}). Within the same week, different letters show significantly different values for P≤0.05 (LSD test). Vertical bars show SE of the means (n=5); NS= not significant.

At the end of the monitoring period, no statistically significant differences were observed between TE-treated plants and control plants in fresh matter production, while dry matter decreased significantly compared to control at 0.2 and 0.8 kg a.i. ha^{-1} but, surprisingly, not at 0.4 kg a.i. ha^{-1} (Table 1).

Table 1 - Aerial biomass production in Patriot bermudagrass treated with different doses of TE (kg a.i. ha-^{1}) seven weeks after treatment

Treatment TE (kg a.i. ha^{-1})	Fresh matter (g per pot)	Dry matter (g per pot)	Number of stolons per pot	Number of sprigs per pot
0	128.5±5.1 ab (z)	38.3± 4.6 a	110±7.4 a	915±83.3 a
0.1	136.7±5.8 a	37.9±1.3 a	104±6.4 ab	912±138.3 a
0.2	113.2±5.2 b	31.8±1.5 b	86±2.6 b	673±48.0 ab
0.4	121.0±4.7 b	34.5±2.6 ab	89±6.3 b	786±80.6 ab
0.8	115.0±6.6 b	31.3±2.0 b	62±5.6 c	597±72.9 b

(z) Values in the same column followed by different letters are significantly different for P≤0.05 (LSD Test).
Values are means (± SE) of five replicates.

TE treatment had a detrimental effect on stolon number per pot starting from the rate 0.2 kg a.i. ha^{-1} upwards, but the average number of nodes derived from one pot decreased significantly only at the highest rate applied (0.8 kg a.i. ha^{-1}) (Table 1).

4. Discussion and Conclusions

The effect of TE on the number of bermudagrass stolons was previously investigated by Fagerness *et al*. (2002). These authors found that TE (0.11 kg a.i. ha^{-1}) did not affect the number of stolons in Tifway bermudagrass when plants were grown in a 22/17°C day/night temperature regime, while at 36/31°C TE-treated plants showed about twice as many stolons as control plants four weeks after TE application. In our experiment, temperature ranged between 20 to 35°C and 0.1 kg TE ha^{-1} did not cause any effect on stolon number in Patriot bermudagrass seven weeks after its application. On the contrary, higher rates of TE (0.2, 0.4 and 0.8 kg a.i. ha^{-1}) reduced significantly the number of stolons compared to control.

An indirect evaluation of the effect of TE on stolon lengthening in turfgrass species may come from percentage lateral regrowth, a parameter used to estimate the horizontal growth or recovery of turfs as described by Bunnell *et al*. (2005). These authors applied TE at 0.039 kg a.i. ha^{-1} to TifEagle bermudagrass every three weeks and measured percentage lateral regrowth weekly for eight weeks. Averaged weekly data of TE-treated plants did not show any differences compared to untreated plants. Totten *et al*. (2006) reported that TE did not affect the percentage lateral regrowth in Tifway bermudagrass when used at the rate 0.052 kg a.i. ha^{-1}, while at 0.104 kg a.i. ha^{-1} percentage lateral regrowth was significantly reduced compared to control two weeks after TE application. In our experiment, a TE rate of at least 0.2 kg a.i. ha^{-1} was needed for a significant decrease of stolon length in Patriot bermudagrass (Fig. 1). That effect lasted for two weeks after TE treatment.

Internode length after one, two or three TE applications at 0.153 kg a.i. ha^{-1} to a mature Tifway bermudagrass turf was measured by Richardson (2002). The measurements were taken ten weeks after the last TE application and no effect of the PGR was detected. In our experiment internode length was significantly shortened in TE-treated plants at any applied TE rate one week after one application; from two to seven weeks after treatment stolon showed average internode length with statistically significant differences between control and TE-treated plants at the rate 0.2 kg a.i. ha^{-1} and higher.

In conclusion, our results suggest that TE may be advantageously used to control stolon development of Patriot bermudagrass for nursery purposes (shortening of the internodes in plants grown in pot to facilitate stolon division practice). In order to obtain prolonged effect, higher rates than those normally adopted in bermudagrass turf management seemed to be required (at least 0.2 kg a.i. ha^{-1}). The highest rate applied (0.8 kg a.i. ha^{-1}) was not advisable as it caused also almost a halving in the number of stolons per pot and a statistically significant decrease in the yield of sprigs. Under the conditions in which we operated, the best result was achieved at the rates 0.2 and 0.4 kg a.i. ha^{-1}. With such rates, although the number of stolons per pot was significantly reduced compared to control, the yield in nodes remained unvaried.

Acknowledgements

This research was funded by the Ministry of Agricultural, Food and Forestry Policies of Italy. Project: "Sistemi avanzati per la produzione vivaistica di tappeti erbosi di specie macroterme ad uso multifunzionale a basso consumo idrico ed energetico".

The authors wish to thank the Pacini Horticultural Nursery for the use of the greenhouses and other facilities.

References

BALDI A., LENZI A., NANNICINI M., PARDINI A., TESI R., 2010 - *Plant growth regulator treatments for the management of Patriot bermudagrass*. - Proceedings of the second Conference of the European Turfgrass Society. Angers, France, April 11-14, pp. 32-34.

BALDWIN C.M., LIU H., MCCARTHY L.B., BAUERLE W.L., TOLER J.E., 2006 - *Effect of trinexapac-ethyl on the salinity tolerance of two ultradwarf bermudagrass cultivars.* - HortScience, 41(3): 808-814.

BEARD J.B., 1989 - *Turfgrass water stress: drought resistance components, physiological mechanisms, and species-genotype diversity.* - Proceedings of the Sixth International Turfgrass Research Conference, Tokio, Japan, pp. 23-28.

BEARD J.B., SIFERS S.I., 1997 - *Genetic diversity in dehydration avoidance and drought resistance within the* Cynodon *and* Zoysia *species*. - Int. Turfgrass Soc. Res. J., 8: 603-610.

BIRAN I., BRAVDO B., BUSHKIN-HARAV I., RAWITZ E., 1981 - *Water consumption and growth rate of 11 turfgrasses as affected by mowing height*. - Agron. J., 79: 85-90.

BUNNELL B.T., MCCARTY L.B., BRIDGET JR. W.C., 2005 - *'TifEagle' bermudagrass response to growth factors and mowing height when grown at various hours of sunlight.* - Crop Sci., 45: 575-581.

CARROW R.N., DUNCAN R.R., 1988 - *Salt-affected turfgrass sites*. *Assessment and management*. - John Wiley & Sons, Hoboken, New Jersey, USA, pp. 185.

COOPER R.B., 2003 - *Summary of 2003 Cutless 50WP turfgrass growth regulator research on 419 bermudagrass fairways.* - SePRO Corp. www.sepro.com/documents/cutless-cooper.pdf.

CROCE P., DE LUCA A., FALCINELLI M., 2006 - *Tappeti Erbosi*. - Edagricole, Bologna, pp. 340.

CROCE P., DE LUCA A., MOCIONI M., VOLTERRANI M., BEARD J.B., 2004 - *Adaptability of warmseason turfgrass species and cultivars in a Mediterranean climate*. - Acta Horticulturae, 661: 365-368.

DUDECK A.E., PEACOCK C.H., 1993 - *Salinity effects on growth and nutrient uptake of selected warm-season turf.* - Int. Turfgrass Soc. Res. J., 7: 680-686.

FAGERNESS M.J., YELVERTON F.H., 1999 - *Effects of trinexapac-ethyl on late season development and cold hardiness of 'Tifway' bermudagrass*. - Proc. Northeast. Weed Sci. Soc., 53: 63.

FAGERNESS M.J., YELVERTON F.H., 2000 - *Tissue production and quality of 'Tifway' bermudagrass as affected by seasonal application patterns of trinexapac-ethyl*. - Crop Sci., 40: 493-497.

FAGERNESS M.J., YELVERTON F.H., LIVINGSTON D.P. III, RUFTY Jr. T.W., 2002 - *Temperature and trinexapac-ethyl effects on bermudagrass growth, dormancy, and freezing tolerance.* - Crop Sci., 42: 853-858.

HARIVANDI M.A., MARCUM K.B., 2008 - *A review of salt tolerance among sports field turfgrasses*. - Acta Horticulturae, 783: 159-162.

JOHNSON B.J., 1994 - *Influence of plant growth regulators and mowing on two bermudagrasses.* - Agron. J., 86: 805-810.

JOHNSON B.J., 1997 - *Growth of 'Tifway' bermudagrass following application of nitrogen and iron with trinexapac-ethyl*. - HortScience, 32(2): 241-242.

LOWE D.B., WHITWELL T., 1999 - *Plant growth regulators alter the growth of 'Tifway' bermudagrass* (Cynodon transvaalensis *x* C. dactylon) *and selected turfgrass weeds*. - Weed Technol., 13(1): 132-138.

MCCULLOUGH P.E., LIU H., MCCARTY L.B., TOLER J.E., 2007 - *Trinexapac-ethyl application regimens influence growth, quality, and performance of bermudagrass and creeping bentgrass putting greens*. - Crop Sci., 47: 2138-2144.

MCCULLOUGH P.E., MCCARTY L.B., LIU H., WHITWELL T., 2005 - *Response of 'TifEagle' bermudagrass* (Cynodon dactylon *x* Cynodon transvaalensis) *to ethephon and trinexapac-ethyl*. - Weed Technol., 19: 251-254.

RICHARDSON M.D., 2002 - Turf *quality and freezing tolerance of 'Tifway' bermudagrass as affected by late-season nitrogen and trinexapac-ethyl*. - Crop Sci., 42: 1621-1626.

ROSSI F., 1993 - *What is it with plant growth regulators?* - The Grass Roots, 21(4): 27.

TOTTEN F.W., TOLER J.E., MCCARTY L.B., 2006 - *'Tifway' bermudagrass growth regulation with the use of trinexapac-ethyl and flurprimidol.* - Weed Technol., 20: 702-705.

VOLTERRANI M., GROSSI N., LULLI F., GAETANI M., 2008 - *Establishment of warm season turfgrass species by transplant of single potted plants.* - Acta Horticulturae, 783: 77-84.

VOLTERRANI M., GROSSI N., PARDINI G., MIELE S., GAETANI M., MAGNI S., 1997 - *Warm season turfgrass adaptation in Italy*. - Int. Turfgrass Soc. Res. J., 8(2): 1344-1354.

VOLTERRANI M., MAGNI S., GAETANI M., DE LUCA A., CROCE P., MOCIONI M., 2010 - *Bermudagrass evaluation trial in Italy.* - Proceedings of the second Conference of the European Turfgrass Society. Angers, France, April 11-14, pp. 223-225.

WATSCHKE T.L., PRINSTER M.G., BRENNINGER J.M., 1992 - *Plant growth regulators and turfgrass management*, pp. 557-588. - In: WADDINGTON D.V., R.N. CARROW, and R.C. SHEARMAN (eds.) *Turfgrass.* American Society of Agronomy, Agronomy Monograph, n. 32. Madison, WI, USA, pp. 805.

WU Y., MARTIN D.L., ANDERSON J.A., BELL G.E., ANDERSON M.P., WALKER N.R., MOSS J.Q., 2009 - *Recent progress in turf bermudagrass breeding research at Oklahoma State University*. - USGA Turfgrass and Environmental Research Online, 8(16): 1-11.

Adv. Hort. Sci., 2012 26(1): 21-24

Effects of silver nanoparticles on *Tecomella undulata* (Roxb.) Seem. micropropagation

M. Aghdaei, H. Salehi[(1)], M.K. Sarmast
Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran.

Key words: multiplication, Rohida tree, SNPs, tissue culture.

Abstract: Plant tissue culture is a reliable tool for conservation and multiplication of many plants, including medicinal plants. *Tecomella undulata* (Roxb.) Seem. is a plant native to tropical regions such as Iran, India and Pakistan; this precious plant which contains lapachol (a strong antiseptic used against jaundice) is an endangered species, therefore its conservation is of prime importance. The aim of this experiment was to evaluate the effects of silver nanoparticles (SNPs) at concentrations ranging from 5 to 80 mg l^{-1} alone or combined with 6-benzyl-amino-purine (BAP) and indoleacetic acid (IAA) on growth properties of *T. undulata* in aseptic condition. Thidiazuron (TDZ) at concentrations from 0.001 to 20 mg l^{-1} was used in proliferation medium of *T. undulata* single nodes; combinations of BAP (from 0.3 to 1.2 mg l^{-1}), and 2,4-dichloro-phenoxy-acetic acid (2,4-D, 0.2 and 0.4 mg l^{-1}) were also used in callus production and in indirect bud regeneration media. Explants were surface sterilized using 10% Clorox for 7-8 minutes. Results indicated that adding of SNPs in MS medium increased the mean number of fresh shoots per explants (MNFS/E), the percentage of explants producing shoots (PEPS) and also plant survival, due to its action on ethylene blockage. TDZ at the concentration of 0.1 mg l^{-1} increased bud proliferation up to two buds per explants, however higher concentration inhibited growth and in some cases caused death of the explants.

1. Introduction

Tecomella undulata (Roxb.) Seem., known as Rohida tree, is an ornamental and medicinal shrub species of the Bignoniaceae family widespread in tropical regions such as Iran, India, and Pakistan. This endangered plant species currently grows in arid and semi-arid regions of southern parts of Iran. Its bark contains lapachol, a naphthoquinone with anticancer, antibacterial, antifungal, antivirus (Consolacao *et al*., 1975; Guiraud *et al*., 1994; Hussain *et al.,* 2007) and analgesic (Ahmad *et al*., 1994) activities. It may have a pivotal role in environmental conservation in the arid parts of Iran; furthermore it is an accepted tree species in agroforestry systems (Tewari and Singh, 2009). Due to its beautiful flowers and semi-deciduous habit, it can be used in landscaping with some limitations due to its sterility and low potential germination of the few seeds produced in Iran. Karami and Salehi (2010) reported that rooting of stem cuttings is limited from spring to autumn in this species and it is included in the list of hard to root woody plant species. Therefore, micropropagation is necessary to protect this endangered species. Plant tissue culture is one of the most important steps in genetic transformation and plant biotechnology studies to produce plantlets from stock plants as a rapid and efficient procedure throughout the year (Giri *et al*., 2004; Sarmast *et al*., 2009). There are few reports about micropropagation of *T. undulata*; *in vitro* seasonal effect on shoot proliferation was reported (Rathore *et al*., 1991). Attempts have been made to multiply *T. undulata* plants through micropropagation. Rathore *et al*. (1991) reported that about 8-10 shoots were obtained over two to three weeks at 31ºC in MS medium supplemented with BAP (2 mg l^{-1}) and IAA (0.05 mg l^{-1}). Robinson *et al.* (2005) claimed that MS basal medium supplemented with 1.5 mg l^{-1} BAP and 0.02 mg l^{-1} IAA was the most effective medium for maximum (95%) regeneration of nodal explants. They reported that 29 shoots per nodal segment were observed on MS medium supplemented with 0.75 mg l^{-1} BAP and 0.01 mg l^{-1} IAA within 3 weeks. Furthermore, they observed about 27% mortality after transfer of plantlets to soil mixture. Aslam *et al*. (2009) developed a transformation protocol for *osmotin* gene in *T. undulata*. The effects of silver nanoparticles (SNPs) on decontamination in tissue culture systems have been reported (Abdi *et al*., 2008; Sarmast *et al*., 2011). However, the effect of silver-based material on ethylene mode of action is not well acknowledged in plants (Taiz and Zeiger, 2006). Explants grown in media supplemented with silver ions (Eapen and George, 1997; Zhang *et al*., 2001) and SNPs (Sarmast *et al*., 2011) were relatively healthier than control. The most important step of any *in vitro* propagation system is mass multiplication of plantlets that are genetically homogenous and phenotypically uniform (Sarmast *et al*., 2012). Therefore, micropropagation is restricted to direct regeneration.

[(1)] Corresponding author: hsalei@shirazu.ac.ir
Received for publication 17 October 2011
Accepted for publication 31 January 2012

The aim of the present work was to assess SNPs as ethylene inhibitor for the improvement of micropropagation in *T. undulata.* A secondary objective was to examine TDZ and BAP effects on direct and indirect regeneration in *T. undulata* through single node and callus obtained from the proximal part of the explants.

2. Materials and Methods

More than 15-year-old Iranian *Tecomella undulata* plants were chosen for the present study. Stem pieces (4 cm long) were cut and prewashed in tap water for 15 min. Explants were then treated with 10% Clorox (containing 5.25% sodium hypochlorite) plus 0.2% household detergent for 7 min for surface sterilization and then rinsed six times with sterilized distilled water. The stem pieces were finally cut into nearly 10 mm long segments (including a single node) and placed with their proximal ends on MS (Murashige and Skoog, 1962) basal medium with 3.0% sucrose and 0.8% agar. Mean number of fresh shoots per explant (MNFS/E), mean length of shoots per explant (MLS/E), mean diameter of callus per explant (MDC/E) and percentage of explants producing shoots (PEPS) on MS medium supplemented with SNPs (5 to 80 mg l^{-1}) and combination of BAP (2.5 mg l^{-1}) and IAA (0.1 mg l^{-1}) (Aslam *et al.,* 2009) along with SNPs (5 to 80 mg l^{-1}) were evaluated. Thidiazuron (TDZ) was used at concentrations ranging from 0.001 to 10 mg l^{-1} for assessment mean number and length of shoots per explant, after two weeks of implementation. The average size of nanoparticles used in this study was 18.5 nm and they were synthesized by nanotechnologies, Inc. (Nanocid Company, Tehran, Iran) (Fig. 1). Callus derived from TDZ primary treatments was cultured for one week on MS hormone-free medium and then transferred to MS medium supplemented with BAP (0.3 to 1.2 mg l^{-1}) in combination with 2,4-D (0.2 and 0.4 mg l^{-1}) for indirect bud formation. The explants were subcultured every two weeks. The pH of the media was adjusted to 5.8 before autoclaving for 15 min at 121°C and 1.5 kg cm^{-2} pressure.

Cultures were kept at 25±2°C under cool white fluorescent light (30 µmol m^{-2} s^{-1}) with 16/8 h photoperiod. The experiment was conducted as a completely randomized design. Means were compared using LSD at p≤0.05 with SPSS software (SPSS Inc., Chicago, USA).

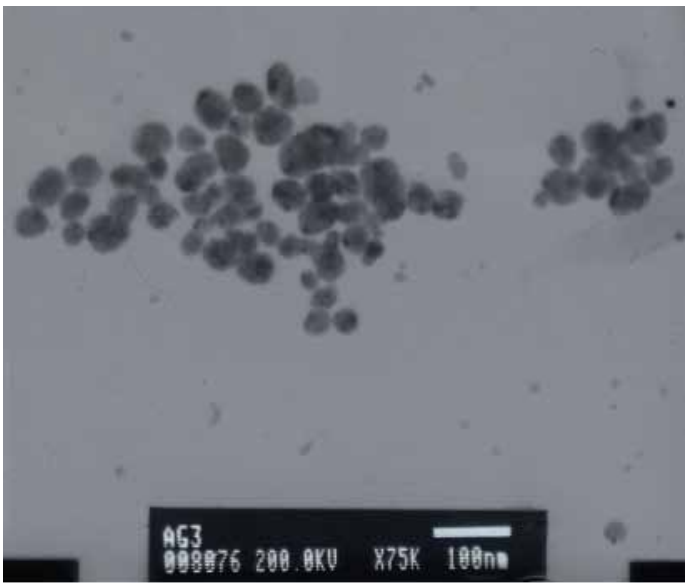


Fig. 1 - Transmission electron microscopy (TEM) micrograph of Ag nanoparticles (scale bar of 100 nm).

3. Results and Discussion

Completely healthy and disinfected explants were achieved following treatment with 10% Clorox for 7 min. Explants cultured on MS basal medium supplemented with different concentrations of SNPs had a higher MNFS/E, MLS/E and PEPS than control (Table 1). In other words, SNPs had positive effects on single node explants of *T. undulata,* but MDC/E in the control was higher than in other treatments. For this reason, there were not significant differences between 10 mg l^{-1} SNPs compared to 80 mg l^{-1} on MNFS/E and MLS/E and, in order to save on cost, the use of 10 mg l^{-1} SNPs in tissue culture of *T. undulata* was proposed. In another experiment (Table 1) there were

Table 1 - Comparing SNPs and combination of SNPs along with BAP (2.5 mg l^{-1}) and IAA (0.1 mg l^{-1}) after two weeks on *T. undulata* (RoxB.) single node explants

SNP (mg l-1)	Mean number of shoots per explants		Mean length of shoots per explants MLS/E		Mean diameter of callus per explants		Percentage of explants producing shoots	
	SNP	SNP+BAP+IAA	SNP	SNP+BAP+IAA	SNP	SNP+BAP+IAA	SNP	SNP+BAP+IAA
0	1.06 cd [z]	1.06 cd	2.03 a-c	2.03 a-c	4.27 a	4.27 a	62 ab	62 ab
5	1.00 c	1.24 b-d	1.81 bc	3.01 ab	0.39 c	5.10 a	50 b	67 ab
10	1.65 ab	0.37 e	3.59 ab	1.07 c	0.75 c	2.10 bc	77 a	27 c
20	1.06 cd	1.16 cd	2.33 a-c	2.92 ab	1.19 c	5.02 a	70 ab	70 ab
40	1.82 a	1.41 a-d	3.74 a	1.94 a-c	2.22 bc	5.17 a	80 a	70 ab
80	1.68 a	1.49 a-c	3.02 ab	2.73 a-c	1.27 c	3.81 ab	85 a	70 ab
Mean	1.37 a	1.12 b	2.75 a	2.28 a	1.68 a	4.24 b	70 a	61 b

[z] In each column, means with the same letters are not significantly different at ≤ 0.05 level of probability using LSD.

not significant effects between explants grown in medium supplemented with BAP (2.5 mg l^{-1}) and IAA (0.1 mg l^{-1}) compared with medium supplemented with different concentrations of SNPs. When we compared data of SNPs with SNPs in combination with plant growth regulators (BAP and IAA), there were significant effects between MNFS/E, MDC/E and PEPS (Table 1). Our results demonstrate that Iranian *Tecomella undulata* (Roxb.) Seem. had high potential for producing callus from stem segments even in hormone-free medium, but after a couple of days they turned brown and died. Even single node explants, after nearly three weeks, had necrotic leaves. The use of common antioxidants such as ascorbic and citric acid or activated charcoal (data not shown), did not have significant effects on survival of single node explants and callus derived from stem segments.

Leaves of *T. undulata* produced callus in MS medium but these, when attached to a single node, directly started callus formation. BAP at the concentration of 0.9 mg l^{-1} allowed regeneration of few buds on callus produced from stem segments (data not shown). Results indicate that TDZ at the concentration of 0.1 mg l^{-1} increased MNS/E and MLS/E up to 2 and 3.33 mm respectively from samples taken in autumn (Table 2, Fig. 2). However TDZ at low concentration had a positive effect on callus production, but concentrations of more than 0.01 mg l^{-1} decreased the dimensions of MDC/E.

Table 2 - Regeneration response of *T. undulata* (RoxB.) single node explants on MS medium supplemented with TDZ after two weeks

SNP (mg l^{-1})	Number of shoots per explants	Mean length of shoots per explants (mm)	Mean diameter of callus per explants (mm)
0.000	0.85 b-d [(z)]	2.28 a-c	4.23 a-d
0.001	0.90 bc	1.66 a-d	4.80 ab
0.010	1.13 b	2.83 ab	5.33 a
0.100	2.00 a	3.33 a	3.46 a-d
1.000	0.53 b-d	1.66 a-d	4.40 a-c
10.000	0.10 cd	0.40 d	0.16 c-d
Mean	0.93	1.98	3.63

[(z)] In each column, means with the same letters are not significantly different at ≤ 0.05 level of probability using LSD.

Fig. 2 - Proliferating single node explants of *T. undulata* in medium supplemented with TDZ (0.1 mg l^{-1}).

As desert ecosystems currently cover about 35% of the earth's land surface and also global warming and water deficiency have become worldwide problems, it is urgent to focus on tolerant plant species in these areas (Hellen, 1991). Utilization of plants for medicinal purposes in Iran has been documented in ancient literature. Avicenna (Abu Ali Sina) was a Persian physician and philosopher whose medical system was for a long time the standard in Europe and in the Middle East (Berman *et al*., 2009). To protect ecosystems from drought, an option is propagation of tolerant plants such as wild medicinal plants and a rapid and efficient technique for plant propagation is micropropagation (Thorpe, 2007). Explants used in the present study were collected in October and we believe their low regeneration potential was due to seasonal effects. TDZ is one of the most active cytokinin-like substance used successfully for regeneration of recalcitrant woody plants (Huetteman and Preece, 1993; Sarmast *et al*., 2012). The present study indicates that BAP along with IAA did not have a high potential to induce bud regeneration, as previously reported (Rathore *et al*., 1991; Robinson *et al*., 2005). The data presented in this work demonstrated that, at increasing SNPs concentrations, the dimensions of MDC/E decreased and that buds derived from callus caused somaclonal variation (Larkin and Scowcroft, 1981; Mondal and Chand, 2002; Sarmast *et al*., 2012). Hence, we conjecture that SNPs may be helpful in micropropagation to avoid indirect regeneration and the connected somaclonal variability. Sarmast *et al*. (2011) reported that *Araucaria excelsa* explants, grown in MS medium supplemented with SNPs, were fresher than in MS medium only and that $AgNO_3$ in *Brassica spp.* significantly affected bud formation on callus induction medium (Akasaka-Kennedy *et al*., 2005) and had positive effects on shoot regeneration in *Brassica oleracea* var. *italica* (Qin *et al*., 2007). Ethylene, the simplest olefin, exists in gaseous state in environmental conditions; it is biologically active in trace amounts and regulates many aspects of the plant life cycle such as senescence (Lin *et al*., 2009; Taiz and Zeiger, 2006). In tissue culture vessels, plantlet growth and development can be severely influenced by gaseous effects, especially of ethylene at elevated level that may damage explants by suppressing their growth and causing hyperhydration (Ziv, 1995). Additionally, SNPs affected ethylene formation and it can be concluded that increasing of durability of explants in culture vessel is due to ethylene blockage. In tissue culture of woody plants, rooting is a severe problem (Giri *et al*., 2004; Sarmast *et al*., 2012), therefore a separate experiment is now in progress to improve rooting of *T. undulata* with mediating *Agrobacterium rhizogenes* (strain K559).

References

ABDI G.H., SALEHI H., KHOSH-KHUI M., 2008 - *Nano silver: a novel nanomaterial for removal of bacterial contaminants in valerian* (Valeriana Officinalis *L.*) *tisue culture*. - Acta Phisial. Plant., 30: 709-714.

AHMAD F., ALAM KHAN R., RASHEED S., 1994 - *Preliminary screening of methanolic extracts of* Celastrus paniculatus *and* Tecomella undulata *for analgesic and anti-inflammatory activities.* - J. Ethnopharmacology, 42: 193-198.

AKASAKA-KENNEDY Y., TOSHIDA H., TAKAHATA Y., 2005 - *Efficient plant regeneration from leaves of rape seed (*Brassica napus *L.): the influence of $AgNO_3$ and genotype*. - Plant Cell Rep.**,** 24: 649-654.

ASLAM M., SINGH R., ANANDHAN S., PANDE V., AHMED Z., 2009 - *Development of a transformation protocol for* Tecomella undulata *(Smith) Seem. from cotyledonary node explants*. - Sci. Hort., 121: 119-121.

BERMAN P., BIANQUIS T., BOSWORTH C.E., VAN DONZEL E., HENRICHS W.P. BRILL., 2009 - *Ibn Sina ("Avicenna")*. - In: BERMAN P., T. BIANQUIS, C.E. BOSWORTH, E. VAN DONZEL, and HENRICHS W.P. BRILL (eds.) *Encyclopedia of Islam*. 2nd edition. Accessed through Brill online: www.encislam.brill.nl

CONSOLACAO M., LINARDI F., OLIVEIRA M.M, SAMPAIO M.R., 1975 - *A Lapachol derivative active against mouse lymphocyte leukemia.* - J. Medicin. Chem., 18: 1159-1161.

EAPEN S., GEORGE L., 1997 - *Plant regeneration from peduncle segments of oil seed* Brassica *species: influence of silver nitrate and silver thiosulfate*. - Plant Cell Tiss. Org. Cult., 51: 229-232.

GIRI C.C., SHYAMKMAR B., ANJANEYLNU C., 2004 - *Progresses in tissue culture, genetic transformation and application of biotechnology to trees: an overview*. - Trees., 18: 115-135.

GUIRAUD P., STEIMAN R., CAMPOS-TAKAKI G.M., 1994 - *Comparison of antibacterial and antifungal activities of Lapachol and beta-Lapachol*. - Planta Medica, 60: 373-374.

HELLEN U., 1991 - *Desertification-time for an assessment?* - AMBIO, 20: 372-383.

HUETTEMAN C.A., PREECE J.E., 1993 - *Thidiazuron: a potent cytokinin for woody plant tissue culture*. - Plant Cell Tiss. Org. Cult., 33: 105-119.

HUSSAIN H., KROHN K., AHMAD V.U., MIANA G.A., GREE, I.R., 2007 - *Lapachol: An overview.* - Arkivoc., 2: 145-171.

KARAMI A., SALEHI H., 2000 - *Adventitious root formation in Rohida* (Tecomella undulata (AM.) *Seem) cutting*. - Prop. Orn. Plant., 10: 163-165.

LARKIN P.J., SCOWCROFT W.R., 1981 - *Somaclonal variation - a novel source of variability from cell culture for plant improvement.* - Theor. Appl. Genet., 60: 197-214.

LIN Z., ZHONG S., GRIERSON D., 2009 - *Recent advances in ethylene research.* - J. Exp. Bot., 60: 3311-3336.

MONDAL T.K., CHAND P.K., 2002 - *Detection of genetic variation among micropropagated tea* Camellia sinensis *(L). by RAPD analysis. - In Vitro* Cell. Dev. Biol. - Plant, 38: 296-299.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bioassays with tobacco tissue cultures*. - Physiol. Plant., 15: 473-497.

QIN Y., LI H.L., GUO Y.D., 2007 - *High- frequency embryogenesis, regeneration of broccoli (*Brassica oleracea *var. italica) and analysis of genetic stability by RAPD.* - Sci. Hort., 111: 203-208.

RATHORE T.S., SIGH R.P., SHEKHAVAT N.S., 1991 - *Clonal propagation of desert teak (*Tecomella undulata*) through tissue culture.* - Plant Sci., 79: 217-222.

ROBINSON R., BIMLENDRA K., BENIWAL S.V., 2005 - In vitro *shoot multiplication of* Tecomella undulata *(SM.) Seem: An endangered tree species.* - Indian J. Plant Physiol., 10: 372-376.

SARMAST M.K., SALEHI H., KHOSH-KHUI M., 2011 - *Nano silver treatment is effective in reducing bacterial contaminations of* Araucaria excelsa *R. Br. var. glauca explants*. - Acta Biol. Hung., 62(4): 477-484.

SARMAST M.K., SALEHI H., RAMZANI A., ABOLIMOGHADAM A., NIAZI A., KHOSH-KHUI M., 2012 - *RAPD fingerprint to appraise the genetic fidelity of* in vitro *propagated* Araucaria excelsa *R. Br. var. glauca plantlets*. - Mol. Biotechnol., 50(3): 181-188.

SARMAST M.K., SALEHI M., SALEHI H., 2009 - *The potential of different parts of* Sansevieria trifasciata *L. leaf for meristemoids production.* - Aust. J. Basic Appl. Sci., 3: 2506-2509.

TAIZ L., ZEIGER E., 2006 - *Plant Physiology*. - Sinauer Assoc. Inc. 4 ed., pp. 700.

TEWARI V.P., SINGH B., 2009 - *Site index model for* Tecomella undulata *(Sm.) Seem. (Bignoniaceae) plantations in a hot arid region of India.* - J. Arid Environ., 73: 590-593.

THORPE T.A., 2007 - *History of plant tissue culture*. - Mol. Biotechnol., 37: 169-180.

ZHANG P., PHANSIRI S., KAERLAS J.P., 2001 - *Improvement of cassava shoots organogenesis by the use of silver nitrate* in vitro. - Plant Cell Tiss. Org. Cult., 67: 47-54.

ZIV M., 1995 - *In vitro acclimatization. Automation and Environmental Control in Plant Tissue Culture*. AITKEN-CHRISTIE J., T. KOZAI, and M.A.L. SMITH (Eds.). Klumer Academic Publisher. Netherlands, pp. 577.

Adv. Hort. Sci., 2012 26(1): 25-31

Crop load manipulation and fruit cracking in sweet cherry (*Prunus avium* L.)

P.F. Measham*, S.A. Bound, A.J. Gracie*, S.J. Wilson***

** Tasmanian Institute of Agriculture, University of Tasmania, Private Bag 98, Hobart, Tasmania, 7001 Australia.*

*** Tasmanian Institute of Agriculture, 13 St Johns Avenue, New Town, Tasmania, 7008, Australia.*

Key words: fruit crop load, incidence of cracking, sweet cherry.

Abstract: Yield loss from rain-induced fruit cracking is a perpetual risk associated with the production of sweet cherries, and is difficult to manage due to the unpredictability of fruit responses to late season rainfall. The aim of this five-year study was to investigate the relationship between fruit crop load and incidence of cracking. The results showed a negative correlation between crop load and incidence of fruit cracking, and it was found in both natural and manipulated crop load trials for all varieties studied and in all seasons assessed. The effect of crop load on final cracking levels are determined post cell division. Results from this study showed that fruit width was positively correlated with cuticular cracking but, contrary to what has been purported in literature, no relationship between concentration of soluble sugars or firmness with the incidence of cracking was found. This study has confirmed that crop load should be a major consideration in orchard practices in developing strategies to manage fruit cracking.

1. Introduction

Cherry fruit size and quality is an important factor in production and sales of sweet cherry fruit (Proebsting and Mills, 1981). Sweet cherry trees are typically upright, vigorous and non-precocious (Lang *et al*., 2004) so orchard management practices focus on achieving high yields of premium quality fruit through balancing reproductive and vegetative growth. Manipulation of the number of fruits (crop load) on the tree, and leaf area, can be used to encourage larger and sweeter fruit through balanced carbohydrate supply and demand (Lang *et al*., 2004; Spayd *et al*., 1986; Whiting and Lang, 2004). However, in many of these studies, yield losses due to cracking have not been presented (Proebsting and Mills, 1981) even when the economic losses due to cracking can be significant (Hanson and Proebsting, 1996). Given that cracking can be induced by internal vascular flow (Measham *et al*., 2009), it is posited that higher crop loads will reduce the incidence of cracking through increased competition between fruit for assimilate supply.

It has been previously hypothesised that higher crop loads increase competition between fruit for carbohydrates and that lower crop loads result in higher assimilate supply for individual fruit (Spayd *et al*., 1986), and that there can be a resultant increase in size (Spayd *et al*., 1986) and concentration of sugars (Proebsting and Mills, 1981). It has also been noted however, that lower crop loads are associated with increased vegetative growth (Kappel, 1991) and that current season's vegetative growth is a strong sink for carbohydrates. Diurnal translocation of sugars from leaves to fruit can be variable (Richardson, 1998), and therefore it is difficult to assess relationships between sugars and cracking as a result of internally supplied excess water.

Cherry fruits are strong sinks (Ayala and Lang, 2008) and it has been noted that removal of spur leaves had little effect on fruit quality because alternative supplies of carbohydrates were sourced (Whiting and Lang, 2004). Fruit and leaf ratio can be manipulated for optimum quality. Two flower buds per spur has been suggested as the ideal (Whiting and Lang, 2004). An interaction between fruit and leaves was also implicated in the development of cracking, in a study by Measham *et al*. (2010), which showed that leaf removal decreased the development of side cracks in cherry fruit during the few weeks prior to harvest. Furthermore, diurnal water potential gradients and evaporative demands on the leaf influenced vascular flow to the fruit demonstrating a local fruit and leaf interaction.

Thus, given that fruit size (Simon, 2006) and sugar levels (Christensen, 1996) have been associated with the development of cracking, and Simon (2006) cites two studies (Bullock, 1952; Way, 1967) that found trees with high loads that showed little cracking within variety, the potential for crop load manipulation to influence fruit cracking warrants investigation. The aim of this present study is to further examine this relationship between crop load and cracking.

Received for publication 23 October 2011
Accepted for publication 3 February 2012

2. Materials and Methods

Plant material

Mature trees, grown on F12/1 rootstock, were used in all field trials. Trials were undertaken from late October to late January during seasons 2005/06, 2006/07, 2007/08 and 2010/11 in two commercial orchards in Huonville and Old Beach, Tasmania (Australia). All orchards were subjected to standard industry management practices. To investigate the effect of crop load on fruit cracking and type, five manipulated crop load trials were undertaken in years with late summer rainfall; Trials 1 and 2 in seasons 2005/06 and 2006/07 respectively, Trials 3, 4 and 5 in season 2010/11. A study of fruit properties from Trial 5 was undertaken. In addition, a survey of natural crop load and fruit properties over three years (2005/06, 2006/07, 2007/08) was performed. The relationship between levels of cracking *in situ* and the cracking potential using the cracking index (Christensen, 1972) was also evaluated.

Manipulated crop load trials

To assess the impact of crop load on crack development, manipulated crop load trials were undertaken on one variety 'Simone' at one site (Huonville) in two seasons: 2005/06 (Trial 1) and 2006/07 (Trial 2). Three further manipulated crop load trials were undertaken on different varieties and sites in one season, 2010/11; on variety 'Sweetheart' at Huonville and Old Beach (Trial 3 and 4 respectively), and on variety 'Regina' at Huonville (Trial 5). These varieties were chosen due to the variety of crack types they had previously displayed in earlier studies (Measham *et al*., 2009) in Tasmania; 'Simone' showed a tendency for cuticular cracks, 'Regina' for side cracks' and 'Sweetheart' for both.

In Trials 1 and 2, treatments included a low, medium and high crop load, which aimed for 2, 5 or 8 fruit per cm^2 trunk cross sectional area (TCSA) respectively in a randomised complete block design with three replicates (whole tree plots). Treatments were applied at pit-hardening during stage II of fruit growth and development which occurred post bloom at 4 weeks after full bloom (4WAFB). Where the high crop load specified could not be reached, natural crop load was determined and used.

In Trials 3, 4 and 5, treatments included a low, medium and high crop load, applied at three different growth stages in a factorial design with five replicates (whole tree plots). Crop load was achieved by thinning each bud to 1, 2 or 4 floral buds per spur and applied pre bloom (PrB) at dormant bud stage, full bloom (FB) and post bloom (PoB) at four weeks after full bloom. In addition, for Trial 3, a sub sample of 30 non-cracked blemish-free fruits were randomly selected from each replicate tree for individual fruit assessments for size, total soluble solids, and firmness. Mean fruit property values were used to assess relationships with crop load, and with the incidence of cracking *in situ*.

In all trials cracking incidence was determined at harvest. Fruits from each treatment were additionally assessed for cracking index (using 50 non cracked fruits per variety). In all manipulated crop load trials, the actual crop load achieved for all trial trees was recorded.

Natural crop load and fruit properties survey

Natural crop load was recorded at harvest over three seasons on three randomly selected whole trees of available varieties which included 'Kordia', 'Lapin', 'Regina', 'Simone', 'Sweetheart', 'Sylvia' and 'Van'. All fruits were harvested and cracking levels recorded. Cracking incidence recorded at harvest was assessed in relation to natural crop load.

In addition, for each season non-cracked blemish-free fruit from each variety was grouped, and a sub sample of 30 non-cracked fruits were selected for individual fruit assessments for size, weight, total soluble solids, stem length and skin thickness. Mean fruit property values were used to assess the relationship with the incidence of cracking *in situ* for each variety.

Also in 2007/08, fruits from available varieties were assessed for cracking with the cracking index immersion method (Christensen, 1972). The relationship between the cracking index and the level of cracking recorded in the field was investigated. Given that this immersion method depends on water uptake across the skin, the impact of stem removal on uptake and the subsequent index value was also investigated using additional sub samples from variety 'Simone' with stems either removed or left intact.

Measurements

Cracking incidence was determined as per Measham *et al*. (2009), but with apical-end cracks and stem-end cracks combined to give a level of cuticular cracks. Measham *et al*. (2010) concluded that these crack types were likely to be induced through the same mode of water uptake. Cracking index was determined using the immersion method developed by Verner and Blodget (1931) as cited in and refined by Christensen (1972).

All fruit were harvested between 7 A.M. and 12 noon and cracking assessments, morphological measurements and laboratory-based measurements were undertaken on the same day as harvest. Climate data for the months preceding and during harvest was obtained from the Australian Bureau of Meteorology Stations at Huonville (situated less than 5 km from the trial site) and at Old Beach using a PM-K208 PM-11 Phytomonitor Weather Station.

Determination of crop load

Prior to treatment application in manipulated crop load trials, tree girth was measured in centimetres at a point 5 cm above the graft union. TCSA was calculated for each tree for the area (A) of a circle using the formula ($A = C^2/4\pi$), where C = circumference (cm) as described in Measham *et al.* (2009). Crop load was determined as total fruit number per TCSA. To determine natural crop load, all fruits from each tree were counted and crop load expressed as number of fruit per TCSA.

Fruit property tests

Fruit size, weight and total soluble solid (TSS) concentration (brix°) were measured as described in Measham *et al*. (2009). Fruit firmness was measured using a Bioworks Inc. Firmtech 2 with values recorded using ControlSoft software. Stem length (mm) was measured using Vernier callipers and skin thickness was recorded microscopically using a Nikon SMZ800 dissecting microscope.

Statistical analyses

To assess the relationship between crop load and cracking incidence, and crop load with mean fruit properties, data were subject to linear regression tests and ANOVA. Interactions between crop load and timing of thinning to desired load were determined prior to assessing main effects. Analysis of proportion data was performed on transformed data in order to meet the assumptions of analysis.

To assess the effect of natural crop load on fruit properties after accounting for variety, mean fruit property data were subject to ANOVA using variety as a fixed factor, and then to ANCOVA (crop load as the covariate) using PROC GLM (SPSS version 17). Unless specified, all results identified as 'significant' are at probability level of 0.05.

3. Results

Manipulated crop load trials

All manipulated crop load trials received rainfall in the three weeks prior to harvest. In 2005/06 and 2006/07 there was a similar amount of rainfall (37 mm and 41 mm respectively). Trials 3 and 4 in 2010/11 experienced 49 mm and 50 mm rainfall respectively and Trial 5 received 42 mm rainfall (differing due to harvest dates).

A negative linear relationship between actual crop load and total cracking incidence was recorded for variety 'Simone' over both seasons (2005/06 and 2006/07) (Fig. 1). The effect was greater in season 2005/06 than 2006/07, as indicated by the significantly greater magnitude of the slope for each crack type (Slope (B) = -4.69 and -0.77 for total and side cracks and -0.69 and -0.30 for cuticular cracks).

In 2010/11 a significant interaction (P = 0.045) between level and timing of crop load on total cracking in variety 'Sweetheart' at Huonville (Trial 3) (Fig. 2), but not at Old Beach (Trial 4). At Huonville (Trial 3), within thinning times, no significant effect of crop load at the dormant (PrB) or full bloom (FB) thinning times was seen, but there was a significant effect on total (P = 0.025) and side (P = 0.029) cracks (but not on cuticular cracks) when

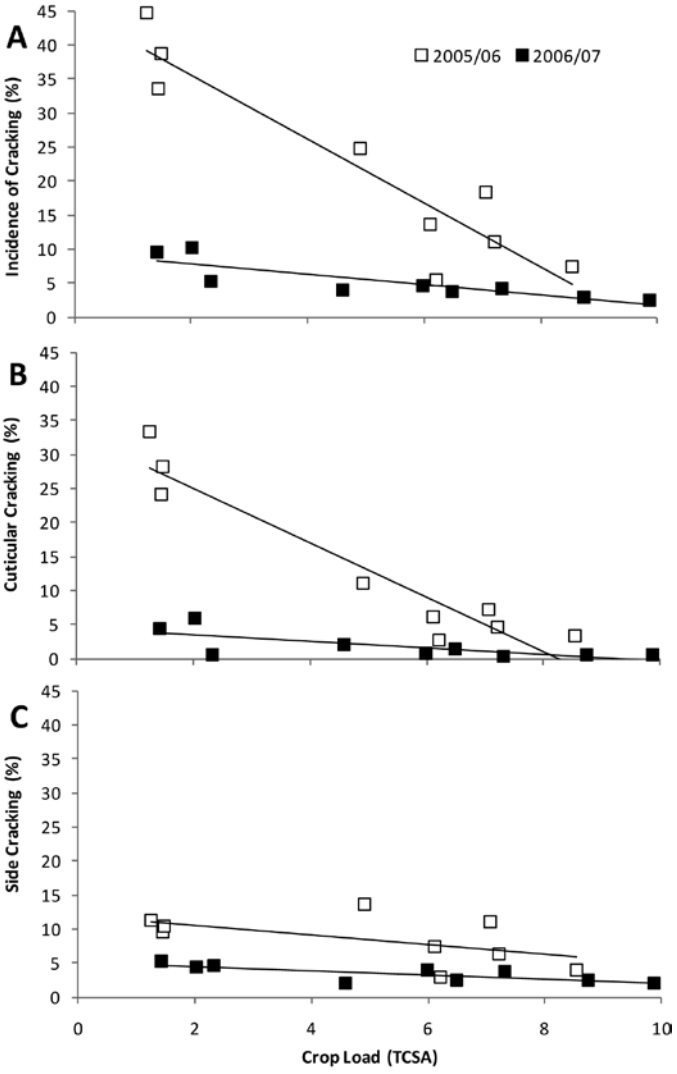


Fig. 1 - Percentage of total cracked fruit (A), cuticular-cracked fruit (B) and side-cracked fruit (C) with actual crop load (TCSA) for variety 'Simone'. Each point is for an individual tree. A significant relationship was found between actual crop load and total cracking incidence in 2005/06 (R^2 = 0.903, P<0.001) and 2006/07 (R^2 = 0.511, P = 0.03), cuticular cracking 2005/06 (R^2 = 0.907, P<0.001) and 2006/07 (R^2 = 0.540, P = 0.02) and side cracking in 2006/07 (R^2 = 0.575, P = 0.02). A significant difference between seasons was found for total cracking and cuticular cracking. Slope (B) of the linear regressions calculated for crop load and cracking incidence were significantly different between years.

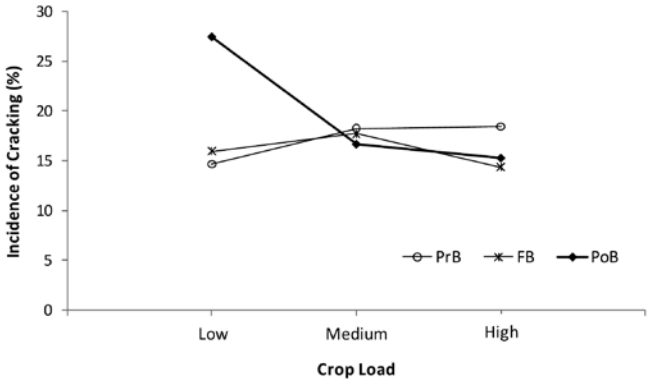


Fig. 2 - Incidence of total cracking at Huonville for variety 'Sweetheart' under low, medium and high crop loads applied at dormant bud stage (PB), full bloom (FB) and four weeks after full bloom (PoB). A significant interaction between crop load level and application timing was evident; low crop load resulted in a significantly higher level of cracking within the post bloom application time only.

thinning was applied post bloom (PoB) (Table 1). High and medium crop load levels set by post bloom thinning resulted in significantly fewer cracked fruit than low crop loads. At Old Beach (Trial 4), there was no interaction between crop load level and timing of thinning; there was, however, a significant main effect of crop load on both total (P = 0.01) and side (P = 0.01) cracks, but not on cuticular cracks. A significant main effect of crop load on total (P < 0.001) and side (P < 0.001) cracks was also seen in variety 'Regina' at Huonville (Trial 5) (Fig. 3).

Table 1 - Incidence of total and side cracking in 'Sweetheart' at Huonville under high, medium and low crop loads applied post bloom

Crack type	Crop load	Cracking incidence
Total	High	15.22 a
	Medium	16.62 a
	Low	27.40 b
Side	High	11.30 a
	Medium	12.44 a
	Low	22.89 b

For each crack type, values followed by different letters indicate a significant difference (P<0.05).

There was an interaction of crop load level and timing of thinning on fruit size in 'Regina' (Fig. 4) where low crop loads resulted in smaller fruit when thinned pre bloom or post bloom. Thinning at full bloom gave smaller fruit with medium loads. No interaction was found for fruit soluble solids or firmness; furthermore, no main effect of crop load or timing was found for fruit firmness. There was a significant main effect of crop load (P = 0.008) and timing of thinning (P = 0.013) on soluble solids with fruit from medium crop loads, and post bloom thinning displaying the highest soluble solids. No fruit properties were correlated with cracking levels except for fruit size, which was positively correlated (P = 0.01) with cuticular cracks only.

Cracking indices determined for all treatments in Trials 3, 4 and 5 are given in Table 2. There was a significant relationship (P = 0.017) between index and total cracking

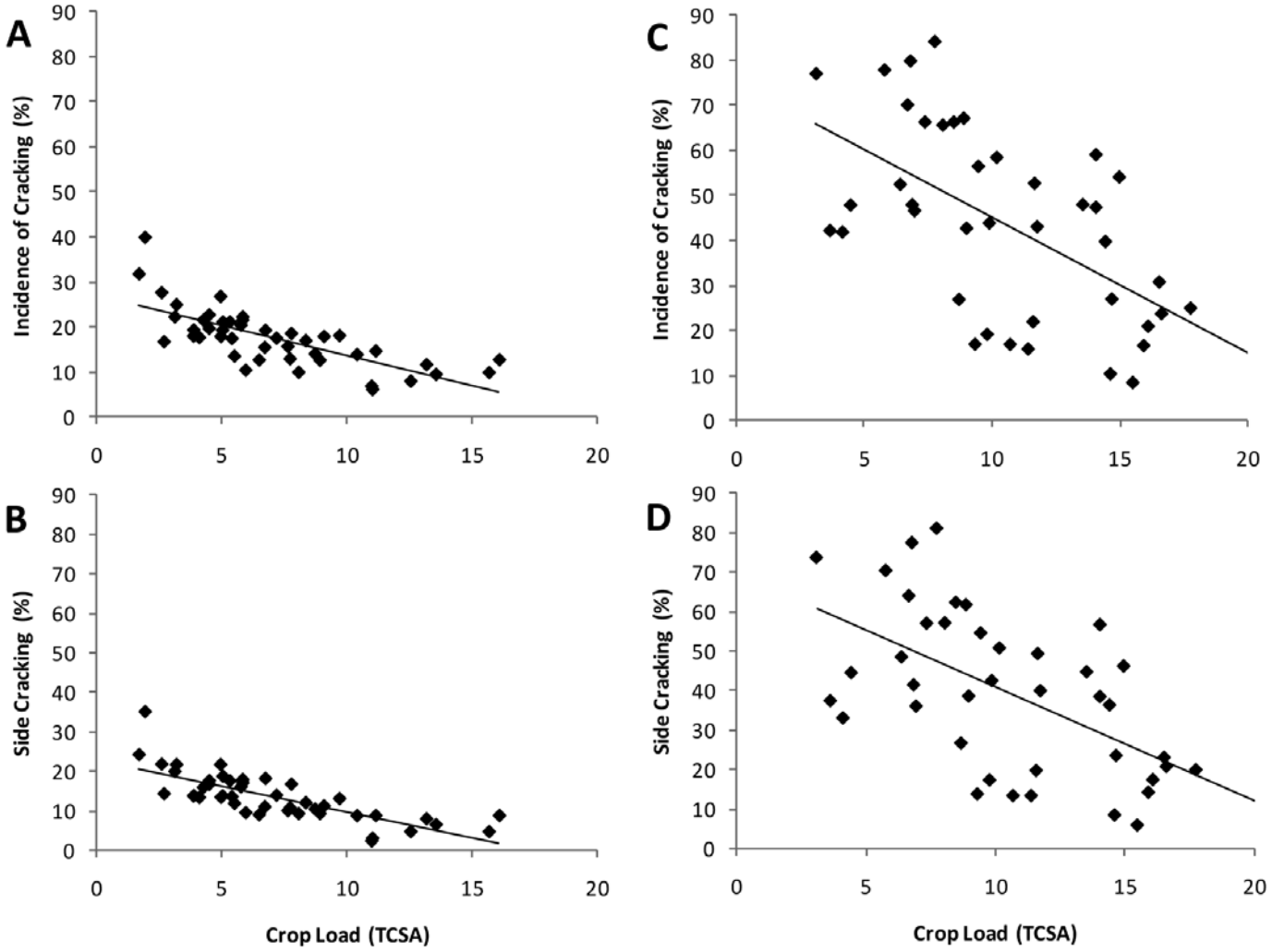


Fig. 3 - Percentage of total cracked fruit (A) (R^2 = 0.69) and side-cracked fruit (B) (R^2 = 0.59) with actual crop load (TCSA) for variety 'Regina' and percentage of total cracked fruit (C) (R^2 = 0.33) and side-cracked fruit (D) (R^2 = 0.32) with actual crop load (TCSA) for variety 'Sweetheart'. Each point is for an individual tree. Significant relationships were found between crop load and total cracking incidence and between crop load and side cracking.

recorded *in situ* for Trail 3 only; no relationship was evident between cracking index and cracking *in situ* for Trial 4 or 5.

Natural crop load and fruit property trials

Lower natural crop loads had higher levels of cracking incidence (Fig. 5). Cracking incidence remained low (less than 5%) for crop loads higher than ten fruit per cm^2 TCSA in all years and for all crack types (Fig. 5). When only using data points of less than 10 fruit per cm^2 TCSA, relationships between cracking and crop load were found to be significant for all crack types in 2005/06 (total, $R^2 = 0.893$, $P < 0.001$; cuticular, $R^2 = 0.853$, $P < 0.001$; side, $R^2 = 0.540$, $P = 0.006$) and for total and cuticular cracks in 2006/07 (total, $R^2 = 0.576$, $P = 0.005$, cuticular, $R^2 = 0.463$, $P = 0.03$).

Across all varieties and seasons no significant relationship was found between any of the fruit property values with total cracking incidence or individual crack type incidence. Little difference can be observed between either weight or total soluble solids and changes in crop load, except perhaps a slight trend in variety 'Sylvia' where a decrease in weight, but not in total soluble solids, occurs with a dramatic increase (three fold to 24 fruit per TCSA)

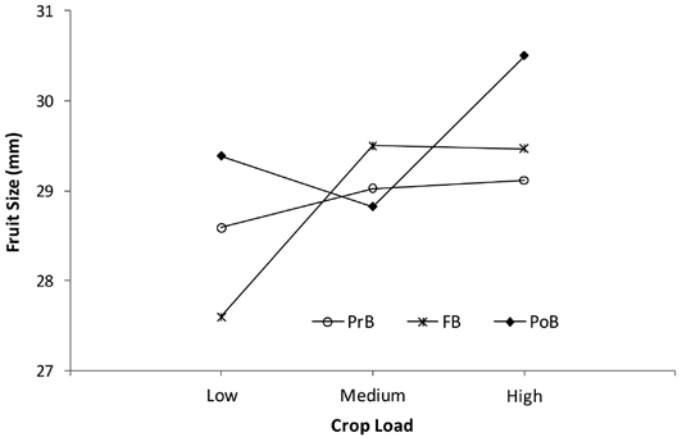


Fig. 4 - Fruit size (mm) determined for fruit from variety 'Regina' under low, medium and high crop loads applied at dormant bud stage (PrB), full bloom (FB) and four weeks after full bloom (PoB). A significant interaction between crop load and thinning time was found.

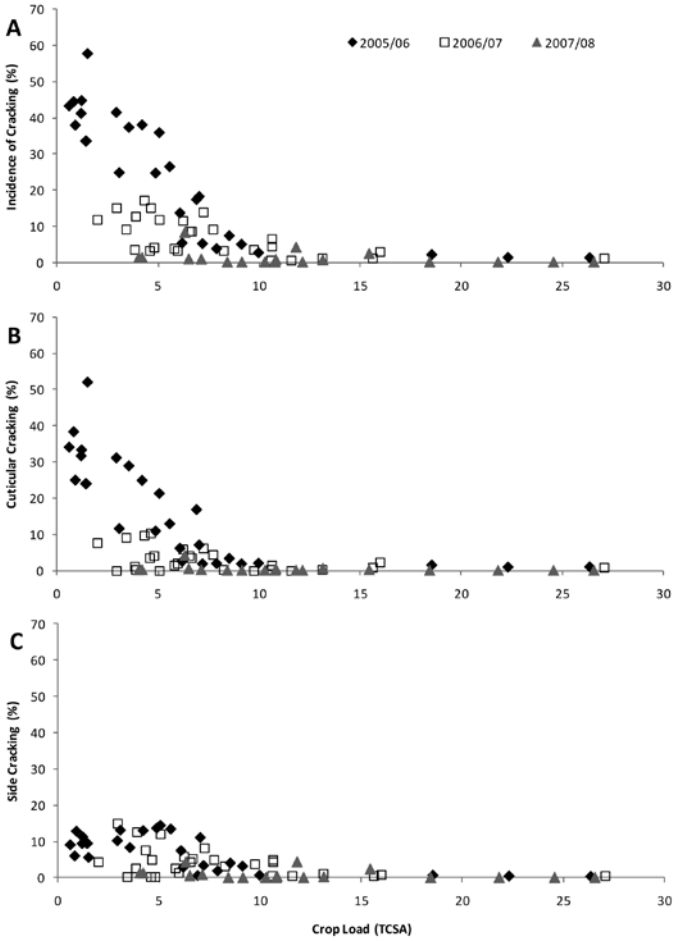


Fig. 5 - The percentage of total cracked fruit (A), cuticular-cracked fruit (B) and side-cracked fruit (C) with natural crop load (TCSA). Each point is for an individual tree. A significant relationship was found between crop load and total cracking incidence in 2005/06 and 2006/07, and between crop load and cuticular cracking in 2005/06, and between crop load and side cracking in 2005/06 and 2006/07.

Table 2 - Cracking index (n = 50) determined for fruit from the three manipulated crop load trials (Trials 3, 4 and 5)

Time of Application	Crop Load	Cracking *in situ* (%)			Cracking index		
		Trial 3	Trial 4	Trial 5	Trial 3	Trial 4	Trial 5
Dormant	High	18	38	7	5	4	24
	Medium	18	32	11	10	6	16
	Low	14	59	28	11	15	21
Full Bloom	High	14	31	6	8	8	3
	Medium	18	39	11	9	7	7
	Low	16	69	26	8	14	7
4WAFB	High	15	28	7	6	9	4
	Medium	17	32	11	9	4	7
	Low	27	50	26	18	24	12

Mean incidence of total cracking is also given.
A significant correlation was found between cracking index and total cracking in Trial 3 only.

in crop load in the third season, 2007/08 (Fig. 6). A significant variety effect was found in fruit weight (P = 0.02), total soluble solids (P = 0.001) and stem length (P < 0.001). After accounting for variety, and analysing data using crop load as a covariate, a significant effect of crop load was found for fruit weight only (P = 0.03).

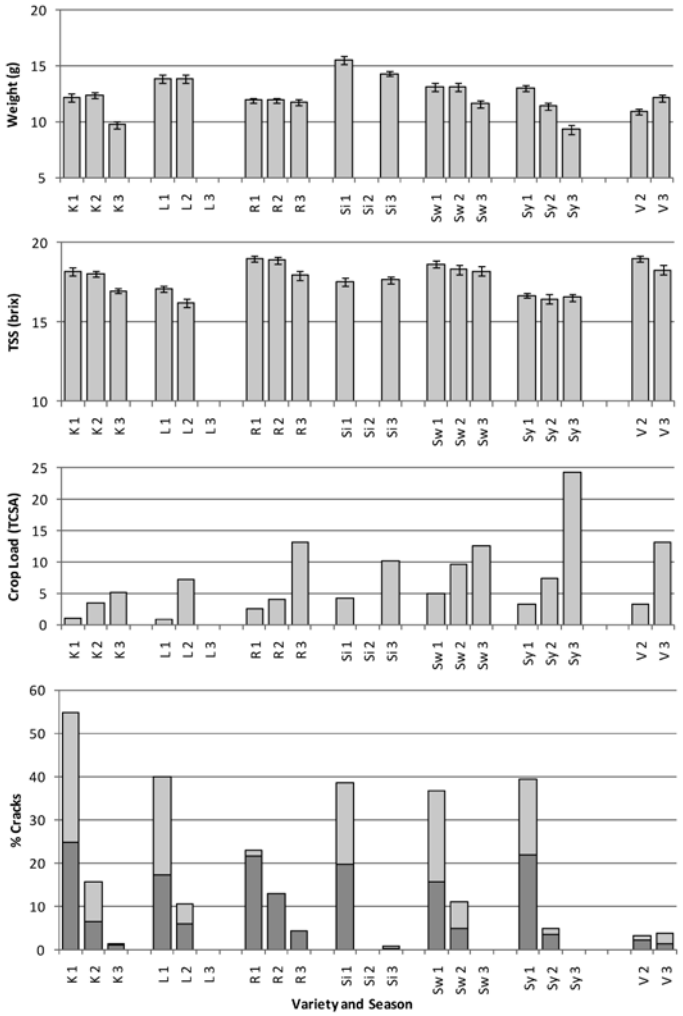


Fig. 6 - Mean fruit weight and total soluble solids for seven varieties over three seasons (error bars represent SEM, n = 30) with mean crop load levels (n = 3) and cracking incidence for the same varieties and seasons. For cracking incidence, expressed as percentage of total yield, columns represent total incidence, where light areas represent incidence of cuticular-cracked fruit, and dark areas represent incidence of side-cracked fruit. Horizontal axis labels represent variety by letter (K - Kordia, L- Lapin, R - Regina, Si - Simone, Sw - Sweetheart, Sy - Sylvia, V - Van) and season by number (1 - 2005/06, 2 -2006/07, 3 2007/08).

A significant relationship between cracking index with total cracking incidence (P = 0.011) and incidence of side-cracked fruit (P<0.001) was found. No significant relationship was found between cracking index and incidence of cuticular-cracked fruit. No differences were found between values for stemless fruit and fruit with stems attached (both had index values of 25).

4. Discussion and Conclusions

Lower crop loads resulted in a greater incidence of fruit cracking in sweet cherry. This was seen in both manipulated crop loads and in the natural crop load survey. Responses to rainfall and the difference in magnitude of cracking between 2005/06 and 2006/07 confirm a strong seasonal impact on crack development, consistent with the findings of Measham *et al.* (2009). However, rainfall *per se* did not account for the differences in cracking between seasons, suggesting that other environmental parameters and fruit growth patterns are also important in the development of cracks.

Fruit development is important in crack susceptibility as all post bloom thinning showed increased cracking with lower crop loads. This implies that the effect of crop load on final cracking levels are determined post cell division, and cracking is therefore more likely to be attributable to cell expansion during the later stages of growth. Cell expansion is a function of internal water entry, which has been linked to increased rates of side-cracked fruit (Measham *et al*., 2010). This also supports the findings of Yamaguchi *et al.* (2002) who linked cracking susceptibility at harvest to cell length. Pre bloom thinning would therefore be the preferable option for manipulating crop load for size whilst minimising the risk of cracking.

In addition, the development of the cuticle during early growth stages should be investigated with regard to cuticle integrity during the later periods of development. The number of cuticular-cracked fruits in low fruit load trees increased significantly in 'Simone'. During cell expansion, relative canopy cover on a whole tree basis in low fruit load trees, compared to high load trees, may prevent moisture loss from the fruit surface through reduced airflow around fruit bunches in a timely and effective manner, confirming the importance of leaf:fruit ratio in quality management decisions (Whiting and Lang, 2004).

In contrast to other studies (Spayd *et al*., 1986; Whiting and Lang, 2004) cracking susceptibility in this study did not seem to be related to fruit quality properties, nor did increased crop load limit fruit size or sugar accumulation, or enhance firmness (Christensen, 1996) in any of the manipulated crop load trails.

In the natural crop load survey, variation in fruit properties was mostly influenced by variety, with crop load only further influencing fruit weight, but not size, sugar level, stem length or skin thickness. Cracking incidence was also not significantly correlated with the fruit properties recorded. This is in contrast with accepted views that both fruit size (Simon, 2006) and sugar levels (Christensen, 1996) are closely linked with cracking. Studies suggesting vegetative growth provides a strong photoassimilate sink in apricots (Costes *et al*., 2000) support these results whereby fruit crop load may not strongly influence source:sink relationships. Results from both the manipulated and natural crop load trials do confirm studies that report no differences in sugar levels between varieties of varying cracking susceptibility (Moing *et al*., 2004).

Furthermore, the results of the present study can be explained by the level of crop load achieved under normal orchard practice. Fruit loads were relatively low in trees from this study with the majority being lower than 15 fruits per cm^2 TCSA; the highest value reached was about 27 fruits per cm^2 TCSA, or the equivalent of just over 2000 fruits on a tree with a trunk circumference of 30 cm. It is possible that fruit quality (size and sugars) was not diminishing under this scenario as there were still available resources within the tree from which to draw. This finding highlights the strong potential for encouraging good fruit set, and subsequent crop load, as a practical and viable management tool in mitigating yield losses from rain-induced cherry fruit cracking, given the significant reduction in cracked fruit with increased crop loads.

The incidence of cracking recorded *in situ* was correlated with the cracking index for varieties in the natural crop load survey but for only one of the manipulated crop load trials. The cracking index procedure may not necessarily be reliable for predicting cracking susceptibility given the differences found in cracking incidence with crop load, with seasons (Measham *et al*., 2009), and when compared to other growing regions (Christensen, 1996*;* Greco *et al*., 2008). The strong correlation between cracking index and the incidence of side cracks recorded *in situ* supports the build up of turgor within the fruit as a likely driver of side cracking (Sekse, 1995), which can be somewhat mitigated by skin and cuticular properties. This could be due to differences in shape; curvature of the skin has been related to cracking susceptibility (Sawada, 1934), and could also explain why size was the only fruit parameter positively correlated with cuticular cracking.

This study has confirmed that crop load management can be successfully used to mitigate cracking without compromising fruit size. The results from this study did not confirm the relationship between fruit size, or sugar, and the incidence or cracking, but highlight the importance of skin properties in crack development.

References

AYALA M., LANG G.A., 2008 - *C-13-photoassimilate partitioning in sweet cherry on dwarfing rootstocks during fruit development*, pp. 625-632. - In: ERIS A., and M. BURAK (eds.) *Proceedings of the V International Cherry Symposium*. International Society for Horticultural Science, Vols. 1 and 2.

CHRISTENSEN J.V., 1972 - *Cracking in cherries. III. Determination of cracking susceptibility*. - Acta Agriculturae Scandinavica, 22: 128-136.

CHRISTENSEN J.V., 1996 - *Rain-induced cracking of sweet cherries: Its causes and prevention*, pp. 297-330. - In: WEBSTER A.D., and N.E. LOONEY (eds.) *Cherries: Crop physiology, production and uses*. CAB International, pp. 513.

COSTES E., FOURNIER D., SALLES J.C., 2000 - *Changes in primary and secondary growth as influenced by crop load in 'Fantasme®' apricot trees*. - J. of Hortic. Sci. & Biotec., 75: 510-519.

GRECO P., PALASCIANO M., MARIANI R., PACIFICO A., GODINI A., 2008 - *Susceptibility to cracking of thirty sweet cherry cultivars*. - Acta Horticulturae, 795: 379-382.

HANSON E.J., PROEBSTING E.L., 1996 - *Cherry nutrient requirements and water relations*, pp. 243-258. - In: WEBSTER A.D., and N.E. LOONEY (eds.) *Cherries: Crop physiology, production and uses*. CAB International, Wallingford, UK, pp. 513.

KAPPEL F., 1991 - *Partitioning of above-ground dry matter in 'Lambert' sweet cherry trees with or without fruit*. - J. Amer. Soc. for Hortic. Sci., 116: 201-205.

LANG G.A., OLMSTEAD J.W., WHITING M.D., 2004 - *Sweet cherry fruit distribution and leaf populations: Modeling canopy dynamics and management strategies*, pp. 591-599. - In: WEBSTER A.D. (ed.) *Key processes in the growth and cropping of deciduous fruit and nut trees*. International Society for Horticultural Science, pp. 715.

MEASHAM P.F., BOUND S.A., GRACIE A.J., WILSON S.J., 2009 - *Incidence and type of cracking in sweet cherry (*Prunus avium *L.) are affected by genotype and season*. - Crop and Pasture Science, 60: 1002-1008.

MEASHAM P.F., GRACIE A.J., WILSON S.J., BOUND S.A., 2010 - *Vascular flow of water induces side cracking in sweet cherry (*Prunus avium *L.)*. - Adv. Hort. Sci., 24(4): 243-248.

MOING A., RENAUD C., CHRISTMANN H., FOUILHAUX L., TAUZIN Y., ZANETTO A., GAUDILLERE M., LAIGRET F., CLAVERIE J., 2004 - *Is there a relation between changes in osmolarity of cherry fruit flesh or skin and fruit cracking susceptibility*? - J. Amer. Soc. for Hortic. Sci., 129: 635-641.

PROEBSTING E.L., MILLS H.H., 1981 - *Effects of season and crop load on maturity characteristics of 'Bing' cherry*. - J. Amer. Soc. for Hortic. Sci., 106: 144-146.

RICHARDSON D.G., 1998 - *Rain-cracking of 'Royal Ann' sweet cherries: Fruit physiological relationships, water temperature, orchard treatments, and cracking index*, pp. 677-682. - In: YSTAAS J., O. CALLESEN (eds.) *Third International Cherry Symposium*, Vols. 1 and 2.

SAWADA E., 1934 - *A physical consideration of the mechanism of the cracking of sweet cherries.* - Transactions of the Sapporo Natural History Society, XII: 365-376.

SEKSE L., 1995 - *Fruit cracking in sweet cherries (*Prunus avium *L.). Some physiological aspects - a mini review*. - Scientia Horticulturae, 63: 135-141.

SIMON G., 2006 - *Review on rain induced fruit cracking of sweet cherries (*Prunus avium *L.), its causes and the possibilities of prevention*. - International Journal of Horticultural Science, 12: 27-35.

SPAYD S.E., PROEBSTING E.L., HAYRYNEN L.D., 1986 - *Influence of crop load and maturity on quality and susceptibility to bruising of 'Bing' sweet cherries*. - J. Amer. Soc. for Hortic. Sci., 111: 678-682.

WHITING M.D., LANG G.A., 2004 - *Effects of leaf area removal on sweet cherry vegetative growth and fruit quality*, pp. 467-472. - In: WEBSTER A.D. (ed.) *Key processes in the growth and cropping of deciduous fruit and nut trees*. International Society for Horticultural Science, pp. 715.

YAMAGUCHI M., SATO I., ISHAGURO M., 2002 - *Influences of epidermal cell sizes and flesh firmness on cracking susceptibility in sweet cherry (*Prunus avium *L.) cultivars and selections*. - J. of the Japanese Soci. for Hortic. Sci., 71: 738-746.

Adv. Hort. Sci., 2012 26(1): 32-38

Influence of quality attributes of early, intermediate and late peach varieties on suitability as fresh-convenience products

F. Colantuono, M.L. Amodio, F. Piazzolla, G. Colelli
Dipartimento di Scienze delle Produzioni e dell'Innovazione nei Sistemi Agro-alimentari Mediterranei (PRIME), Università degli Studi di Foggia, Via Napoli, 25, 71122 Foggia, Italy.

Key words: clingstone peaches, fruit puree, harvest season, mechanical damage, nectarines, sensorial analysis, soluble solids.

Abstract: Fresh convenience products represent a category of minimally processed fruit and vegetables (chunks, mousse, smoothies) that respond to the changes in consumer attitudes. Thanks to the image of convenience (time-saving, snack sizes, no waste, smart packaging) and healthiness their sales are steadily increasing. In this study 26 varieties (including peach, nectarines, and clingstone peaches) from Apulian production were divided into three groups according to harvest dates in early (A), middle (B) and late (C) maturing. Physical, chemical and sensorial analyses were performed in order to select high quality fruits for minimal processing according to the harvesting season. A multivariate Principal Component Analysis was applied to discriminate different varieties for quality attributes. Within Group A, 'Honey Kist' showed the lowest acidity and intermediate susceptibility to mechanical damage. For Group B, 'Stark Red Gold', 'Zee Glo' and 'Venus' resulted different in sensorial evaluation, while 'Loadel' and 'Eolia' were more susceptible to browning. For Group C, 'Tardi Belle' and 'Baby Gold7', although more sensitive to mechanical damage, were differentiated for flavor. Results of this work confirm the extreme variability among varieties in terms of sensorial quality, susceptibility to browning and to mechanical damage, and the importance of assessing varietal screening for selection of most suitable varieties for minimal processing.

1. Introduction

Changes in consumers' social environment represent a constraint to vegetable and fruit consumption and lead to convenience orientation (Candel, 2001). A wide assortment of minimally processed vegetables and fruits (chunks, mousse, smoothies) has been developed to meet consumer needs for ''quick'' and convenient products, and to benefit from the healthy image of fruit and vegetables (Ahvenainen, 1996). One of the main factors that influences quality of fresh-cut products is the enzymatic browning that occurs on product surfaces after cutting (Garcia and Barret, 2002). Thus, cultivar study should be aimed at identifying cultivars which are less susceptible to browning, but also have higher nutritional and sensorial quality, covering all the season, and lead to consumer satisfaction and repeat purchase. Cultivar survey represents an important step when developing a new product (Cabezas-Serrano *et al*., 2009 a, b) allowing identification of genotypes that better respond to postharvest handling and to minimal processing.

Peaches and nectarines are nutritionally important because they contain meaningful amounts of carotenoids including β-carotene (especially yellow-fleshed fruits), lutein and β-cryptoxanthin (Gil *et al*., 2002). Although their availability is limited by season they are one of the most important fruit commodities consumed worldwide (Cantin *et al.,* 2009). Many studies on minimally processed vegetables and fruits focus on microbiological quality, safety, processing and packaging issues (Foley *et al*., 2002; Amodio and Colelli, 2008) but still little information is available on varietal susceptibility. In this light, the aim of the present work was to investigate the suitability of peach varieties to be processed as fresh-convenience products throughout the peach season.

Received for publication 29 September 2011
Accepted for publication 22 February 2012

2. Materials and Methods

From June to September 2010, 26 varieties of peach fruits from the Apulia region (Italy) were collected, including peaches (*Prunus Persica* L.), nectarines (*Prunus Persica* L. var Nectarina) and clingstones peaches (*Prunus Persica* L. Batsch). Based on the 'Redhaven' peach maturing date, peach fruits were divided into three groups: early maturing (Group A), middle maturing (Group B) and late maturing (Group C), as shown in Table 1. Fruits were harvested at a commercial maturity stage, typical for each cultivar, as established by the growers. Peach fruits were then transported to the Postharvest laboratory (University of Foggia)

and kept under controlled temperature and humidity (5°C and 95% RH) for one day before processing. Fruits were washed in a NaOCl solution (100 ppm), rinsed in clear water and then gently dried with a paper tissue. Twenty fruits were used to assess mechanical damage, while the remaining fruits were divided into three replicates of 20 fruits and used to evaluate quality attributes. Physical parameters were evaluated mainly on the whole fruit (with the exception of puree viscosity), while chemical and sensorial analysis were performed on the fruit puree.

Table 1 - Peach varieties grouped in Peach, Nectarine and Clingstone

Peach type		
Peach	Nectarine	Clingstone
Royal Glory	Big Bang	Loadel
Prince Diamond	Big Top	Eolia
Red Elegance	Honey Kist	Baby Gold7
Rome Star	Amiga	
Marilyn	Bigi Lara	
Zee Lady	Ambra	
O'Henry	Laura	
Tardi Belle	Spring Bright	
	Fire Top	
	Maria Camilla	
	Zee Glo	
	Guerriera	
	Venus	
	Stark Red Gold	
	Lidy Star	

Physical and chemical analysis

For each variety the resistance to mechanical damage, in particular the susceptibility to impact bruising, was assessed on 20 fruits. Fruits were individually impacted on one side from a fixed height (30 cm) using a free-falling steel ball (40 g and 21.4 mm diameter), and held at room temperature for 48 hr before measuring. The extension and depth of the bruise (in mm) after peeling was measured. In addition, for each variety the incidence of mechanical bruising (%) was calculated as the ratio between the number of fruits damaged and the total number of fruits considered.

Peach fruit firmness was assessed for each replicate, measuring the force (in N) required by a 8-mm probe to penetrate the peeled surface in two opposite regions of the fruit mesocarp, using a digital penetrometer (TR, Italy).

Flesh fruit color was measured with a colorimeter (CM 2600d, Konica Minolta, Japan) in the CIE L*a*b* mode, taking two measurements per fruit after removing the peel. Hue angle (h°) was calculated as arctg b*/a*.

In order to carry out analysis on the fruit puree, fruits from each cultivar were divided into three groups of 15 fruits each, corresponding to three replicates and from each replicate three subsamples were analyzed. Fruits were peeled, de-stoned, cut into big chunks and blended for 90 s. The purees were promptly transferred to sealed glass jars and stored at 5°C.

A few drops of peach puree were used to measure the total soluble solids content (TSS) with a digital hand refractometer (Atago, Japan). A small amount of fruit puree was transferred to a falcon tube and centrifuged with a centrifuge (PK 121R, Thermo Electron Corporation, France) at 4°C for 5 min. Five grams of surnatant were then used to measure the titratable acidity (TA), with an automatic titrator (TitroMatic 1S, Crison, Spain) measuring the volume of NaOH 0.1N to reach a final pH of 8.1. Results were expressed as percent of citric acid referred to the juice. At the same time, the pH was also measured for each puree sample.

To determine the dry matter content, puree samples were desiccated in an oven at 105°C up to constant weight and the dry matter content was calculated as difference from initial weight.

Peach fruit puree viscosity was determined on samples at 20°C by means of a consistometer (Bostwick, USA); the final results were expressed in cm * 30 s^{-1}.

Sensorial analysis

Sensorial evaluations were carried out on the puree samples, kept at 5°C, within 3 hr after processing. Puree of each variety was distributed into clear plastic cups labeled with a three-digit code. The sensorial test was performed in a sensorial laboratory with 10 trained panelists. To evaluate the intensity of aroma, freshness, sweetness and sourness, an hedonic scale, from 1 to 5, labeled 1=*less intense* to 5=*very intense*, was used. For overall quality the scale reference was 1= *really poor* and 5= *excellent*. For the level of browning, scale reference was 1= *severe browning* and 5= *not browned*.

Statistical analysis

Standard deviation was calculated on mean values for each quality attribute. Principal Component Analysis (PCA) was performed on the data. The biplot technique was used to display the relative positioning of quality attributes and cultivars according to the first two PCs.

3. Results and Discussion

Analysis of fruits in terms of physical, chemical, and sensorial attributes, revealed significant variation among the cultivars.

Evaluation of physical and chemical attributes

The resistance to mechanical damage showed a different incidence among the cultivars. Percentage of incidence to mechanical damage is shown in Table 2: it was not related to the fruit harvest dates but only to the cultivar, even if cultivars in Group B seemed to be more resistant to mechanical damage than the others. Within Group A, most

of the varieties showed a bruise incidence around 55%; 'Amiga' was the most damaged variety (73.7%) whereas 'Spring Bright' did not show any evidence of damage. Within group B only the clingstone peach 'Loadel' showed a high bruise incidence (80%) whilst for the other varieties the average incidence was less than 30%. In Group C all the varieties evaluated were characterized by a high bruise incidence, especially for 'Tardi Belle' (100%).

No differences among the cultivars were found in bruise depth (data not shown) and only the results related to bruise extension are reported. Cultivars from Group A showed a great variability (Table 2), while in Group B the average value of bruise size was 6 mm with the exception of 'Guerriera' and 'Marilyn' that showed the highest (9 mm) and the lowest (2 mm) values, respectively. Within Group C, 'Baby7' and 'Tardi Belle' showed the same bruise size, which was significantly higher than that of 'O'Henry'. Results confirmed that bruise size and incidence of mechanical damage does not correlate with flesh firmness, as reported by Mitchell and Kader (1989). In fact, within the range of commercial maturity studied (Table 2), varieties with the same firmness (i.e. 'Big Bang' and 'Spring Bright') showed different response to mechanical damages (55 and 0% respectively), confirming the great variability within the same peach type and among cultivars.

Fruit flesh color, expressed as hue angle value (Table 2), was not statistically different among cultivars in Groups B and C, while in Group A 'Royal Glory' presented a significantly greener value (hue angle greater than 90°, in the second quadrant of L*a*b* color space) than 'Bing Bang', 'Amiga', 'Ambra', and 'Laura', although showing a very low flesh firmness and acidity.

Fruit puree viscosity was different among the studied varieties; values ranged from 1.5 (in 'Eolia') to 9 cm * 30 s^{-1} (in 'Ambra'), with the most frequent values between 4 and 5.5 cm * 30 s^{-1} (Table 2). The high variability among cultivar viscosity was related to the maturity level; indeed in some cases it seemed influenced by fruit firmness. Group A presented cultivars with higher values compared

Table 2 - Chemical and physical parameters evaluated on peach, nectarine and clingstone peach varieties for Group A (early maturing), Group B (middle maturing) and Group C (late maturing)

Ripening group	Cultivar	Bruise incidence %	Bruise extension (mm)	Firmness (N)	Hue angle (°)	Viscosity (cm*30 s^{-1})	pH	TA (% citric acid)	TSS (°Brix)
A	Big Bang	55	6±0.1	37.6±6.7	75.9±6.4	4.4±0.1	3.77±0.04	0.55±0.70	8.7±1.6
	Big Top	50	11±0.5	43.3±9.1	85.5±8.1	4.6±0.1	3.65±0.13	0.75±0.15	11.7±0.9
	Honey Kist	36	8±0.4	28.0±10.1	87.7±8.2	7.4±0.3	4.00±0.09	0.65±0.09	16.3±2.4
	Amiga	74	10±0.5	19.5±9.0	71.6±11	6.5±0.3	3.37±0.21	1.22±0.13	10.6±0.1
	Bigi Lara	50	12±0.3	25.4±7.7	86.4±5.4	7.5±0.1	3.55±0.03	1.00±0.09	10.9±0.3
	Ambra	60	7±0.2	21.0±7.6	77.8±7.9	9.1±0.5	3.57±0.15	1.19±0.08	10.9±0.3
	Laura	29	4±0.4	18.6±8.2	79.8±3.5	8.4±0.3	3.63±0.09	1.03±0.06	10.4±0.4
	Spring Bright	0	0±0.0	37.9±10.5	83.2±4.4	5.5±0.2	3.69±0.16	1.18±0.20	11.1±0.8
	Royal Glory	65	11±0.3	18.8±9.5	91.4±5.0	3.5±0.2	3.85±0.10	0.68±0.11	9.9±1.0
	Fire Top	25	3±0.1	31.2±11.0	82.8±10	3.4±0.2	3.50±0.06	1.41±0.13	11.7±0.5
B	Maria Camilla	11	6±0.2	25.9±9.1	87.7±6.0	4.6±0.3	3.39±0.05	1.21±0.16	10.2±0.9
	Zee Glo	25	6±0.1	16.8±4.3	85.4±3.8	7.1±0.2	3.43±0.03	1.32±0.38	11.4±1.2
	Guerriera	22	9±0.5	13.2±5.7	82.1±5.7	5.1±0.1	3.49±0.05	1.08±0.10	12.1±1.0
	Diamond Princess	15	8±0.3	16.5±7.2	81.7±4.1	4.8±0.1	3.38±0.13	0.88±0.08	11.7±0.5
	Red Elegance	25	6±0.3	61.9±13.9	84.1±2.7	2.1±0.1	3.44±0.02	0.93±0.08	12.2±0.4
	Rome Star	0	0±0.0	40.7±11.6	81.7±4.2	4.3±0.2	3.52±0.03	0.88±0.03	12.3±0.4
	Loadel	80	6±0.3	32.7±6.0	83.1±4.5	4.5±0.1	3.59±0.06	0.76±0.08	13.4±0.9
	Eolia	25	5±0.2	42.1±14.2	79.0±4.3	1.5±0.2	3.81±0.14	0.77±0.06	12.2±0.4
	Venus	20	6±0.4	37.4±13.2	80.2±5.3	4.1±0.1	3.35±0.07	1.25±0.15	13.3±0.6
	Stark Red gold	20	9±0.8	17.3±4.5	71.4±10	5.5±0.2	3.38±0.04	1.24±0.05	13.5±0.6
	Lidy Star	30	4±0.2	13.0±3.10	78.4±4.8	5.1±0.2	3.76±0.10	0.82±0.06	13.0±0.5
	Marilyn	6	2±0.0	27.6±12.7	82.7±8.2	4.0±0.3	3.53±0.05	0.96±0.13	13.5±0.6
	Zee Lady	20	4±0.2	25.8±10.6	81.5±2.7	3.5±0.2	3.46±0.07	0.96±0.09	13.1±0.3
C	O'Herny	64	4±0.2	62.6±8.7	80.3±4.1	7.0±0.3	3.43±0.06	0.78±0.03	13.4±0.7
	Tardi Belle	100	5±0.1	42.9±9.3	81.6±4.3	7.5±0.1	3.59±0.07	0.69±0.08	12.8±0.4
	Baby Gold 7	87	5±0.1	41.5±5.3	78.6±3.5	4.0±0.3	3.67±0.06	0.52±0.03	12.9±1.2

Mean values±standard deviation.

to the other two groups, in particular 'Ambra' and 'Laura' (9.1 and 8.4 cm * 30 s^{-1} respectively) that were characterized by a low flesh firmness (21 and 18.6 N), whereas 'Red Elegance' that showed low viscosity (2.1 cm * 30 s^{-1}) had a high flesh firmness (61.9 N). Viscosity is an important technological parameter for the formulation of smoothies and fruit purees, due to its influence on product smoothness (or thickness) which may have an effect on the mouth feel of the product.

As for chemical attributes (Table 2), within Group A 'Amiga' showed the lowest pH value (pH 3.37) that was significantly different from 'Honey Kist' (pH 4), which indeed were characterized by a different TA: higher for 'Amiga' (1.22% citric acid) and lower for 'Honey Kist' (0.65% citric acid). In terms of total soluble solids (TSS), 'Honey Kist' showed the highest soluble solid content (16.3°Brix) even though its flesh firmness was similar to the other cultivars. The high TSS value confirms its non-acid characteristics, showing the highest TSS:TA ratio as well (25); this ratio is commonly used as a quality index because it is related to taste perception (Byrne *et al*., 1991). Other researchers (Liverani *et al*., 2003) indicated that the TSS:TA ratio at commercial harvest in non-acid cultivars is three to four times higher than in acid cultivars. 'Royal Glory' showed high pH value (pH 3.85) together with a low TA (0.68% citric acid) and low TSS (9.9 °Brix); this cultivar was significantly different from 'Fire Top' and 'Bigi Lara' that had similar pH values (pH about 3.5) and similar TSS contents (11.7 and 10.9°Brix respectively) but a different TA content (1.41 and 1.00% citric acid respectively). Peach fruit acidity is controlled by several factors such as the cultivar, environmental conditions, canopy position, crop load and fruit maturity (Crisosto *et al*., 1997; Castellari *et al*., 2006). Cultivars with the same flesh firmness as 'Spring Bright' and 'Big Bang' showed similar pH values (pH 3.69 and 3.77 respectively) but different TA values (1.18 and 0.55% citric acid) and TSS (11.1 and 8.7°Brix); this implies that at harvest not only the firmness but also the other chemical attributes must be taken into account to select the right maturity stage for harvesting.

Within Group B, 'Maria Camilla' resulted different from the other cultivars since it showed a lower TSS content (10.2°Brix) with pH 3.39 and high TA (1.21% citric acid). Even if it was in the range of maturity for consumption (firmness 25.9 N) it would have had a low potential impact on consumer preference due to the very low TSS:TA ratio (8.4). 'Eolia' and 'Lidy Star' showed the highest pH values (pH 3.81 and 3.76 respectively) and they were similar in TA (0.77 and 0.82% citric acid) and TSS (12.2 and 13°Brix). 'Eolia' was significantly different from 'Venus' and 'Stark Red Gold' that had lower pH together with higher TA (1.25 % citric acid) and same TSS (13.3 °Brix). With regard to harvest date, it has been reported that medium and late season cultivars have a greater capacity to accumulate sugars compared to early season cultivars, and this is due to the non-interruption of the growing process, sugar accumulation, acid degradation and aroma synthesis (Byrne, 2002). Among late maturing varieties (Group C), 'O'Henry' showed lower pH value (pH 3.43) than the others, indeed it also had higher TA (0.78) than 'Baby Gold7' and no differences in terms of TSS were found among them. The lowest TA value in 'Baby Gold7' gave a higher TSS:TA ratio value (24.8, data not shown) with a potential high consumer preference.

Dry matter includes both soluble (largely sugars) and insoluble solids (mainly the structural carbohydrates and starch). As a large proportion of the dry matter at harvest is starch plus soluble sugars, its value can be related to the soluble sugars that will be contained in the ripe fruit. Indeed in accordance with TSS values, dry matter contents ranged between 8.6% for 'Big Bang' and 16.4 % for 'Honey Kist'. Burdon *et al*. (2004) proposed dry matter in kiwifruit as being both a maturity indicator for timing harvest and also as a predictor of the sensory quality of the fruit once ripe. Results obtained in the present study on peach fruits were in accordance with this theory since peach fruit cultivars ('Honey Kist', 'Lidy Star', 'Stark Red Gold', 'Baby Gold7') with high dry matter content showed a higher value of TSS and were also preferred for sweetness by panelists during sensorial tests.

Sensorial analysis

It is well documented that in peach organic acids and soluble sugars are the major determining factors of fruit taste and, together with the volatiles (responsible for the aroma), have an impact on the overall eating quality of the fruit (Iglesias and Echeverría, 2009). Among the cultivars tested, 'Honey Kist' was the most preferred from Group A together with 'Big Bang', 'Laura' and 'Ambra' as indicated by the overall evaluation score (Table 3). The puree obtained with 'Honey Kist' was described as sweet and fresh, and received a high overall evaluation (score 4.4). Varieties in Group B showed differences for aroma, freshness, sweetness, and overall evaluation: 'Zee Glo', 'Guerriera', 'Diamond Princess', 'Stark Red Gold', 'Venus', 'Lidy Star' and 'Loadel' resulted the most pleasant with a score between 3 (intense or fair) and 4 (good). Moreover, 'Maria Camilla', 'Zee Glo', 'Diamond Princess', and 'Stark Red Gold' were evaluated positively in terms of color, with a score of 3.5 (slightly browned). In Group C 'Tardi Belle' and 'O'Henry' were evaluated well balanced on freshness and aroma while 'Baby Gold7' was considered the sweetest, most probably because of its high TSS:TA ratio.

In general, panelists disliked those varieties that were less sweet and more sour, rating them negatively (score 2-2.5) since TA plays an important role at low TSS levels (<10%). When TSS and TA are low even with a high TSS:TA ratio ('Royal Glory'), the perception of sweetness is low, as reported by Crisosto *et al*. (2006). Moreover, in the selection of new varieties, low acid content (non-acid) and a sweet taste are desirable traits, which give an acceptable flavor and result in better quality for consumers (Nicotra and Conte, 2003).

The nectarine 'Honey Kist', a new variety, was the most appreciated due to its TSS content (16.4°Brix), higher than

the optimum level (11-12%) suggested by Hilaire and Mathieu, (2004) for consumer satisfaction.

Principal component analysis

Each sample from Groups A and B was plotted using the first and second PC factors, which retained 69% of the total variance, while in Group C the first and second PC factors retained 99% of total variance, but in this case only three cultivars were used. Grouping of component loadings separated quality attributes into three groups for all maturing Groups (well displayed by the biplot graphs in figure 1).

Table 3 - Sensorial parameters evaluated on purees of peach, nectarine and clingstone peach varieties for Group A (early maturing), Group B (middle maturing) and Group C (late maturing)

Ripening group	Cultivar	Color	Aroma	Freshness	Sweetness	Sourness	Overall evaluation
A	Big Bang	3.7±0.5	4.4±0.8	4.3±1.1	2.6±0.8	2.9±0.9	3.0±0.8
	Big Top	1.4±0.5	2.6±0.8	2.0±0.7	2.8±0.8	1.8±0.4	2.8±0.8
	Honey Kist	2.4±0.9	3.8±0.8	4.2±0.8	4.0±0.0	1.6±0.5	4.4±0.5
	Amiga	4.2±0.8	3.6±0.5	3.2±0.4	1.6±0.5	3.4±1.5	2.4±1.0
	Bigi Lara	2.0±1.0	3.2±0.8	2.8±0.8	2.4±0.9	1.8±0.8	2.4±0.9
	Ambra	5.0±0.0	4.4±0.9	3.6±0.9	2.2±0.8	3.2±0.4	3.2±0.8
	Laura	4.2±0.4	4.2±0.4	3.0±0.7	2.4±0.9	3.4±0.9	3.0±1.2
	Spring Bright	2.2±0.8	2.8±0.4	2.8±1.3	2.4±0.9	3.4±1.5	2.2±0.8
	Royal Glory	2.0±0.0	3.6±0.5	3.6±0.5	2.0±0.7	3.2±0.4	2.4±0.5
	Fire Top	1.5±0.5	2.6±1.0	2.8±1.0	2.6±1.5	2.7±1.2	2.3±0.9
B	Maria Camilla	3.5±1.5	3.3±0.9	3.3±1.0	3.0±1.2	2.8±0.6	3.5±0.8
	Zee Glo	3.5±1.5	3.3±0.9	3.3±1.0	3.0±1.2	2.8±0.6	3.5±0.8
	Guerriera	1.6±0.5	3.7±1.0	3.2±0.7	3.4±0.9	1.8±0.7	3.3±0.9
	Diamond Princess	3.5±0.4	3.1±1.1	3.5±0.8	2.7±0.5	3.0±1.0	3.1±0.6
	Red Elegance	1.7±0.8	2.1±0.8	2.5±1.4	2.4±0.5	2.8±0.8	2.4±1.1
	Rome Star	1.3±0.5	2.2±0.7	2.8±0.4	2.5±0.8	2.3±1.0	2.8±0.8
	Loadel	2.2±0.7	3.5±0.5	3.2±1.5	3.3±0.4	1.9±0.7	3.6±0.5
	Eolia	1.2±0.4	2.5±0.8	2.8±1.2	3.4±0.6	2.0±0.7	3.2±0.7
	Venus	2.7±1.0	2.7±0.8	2.9±1.0	2.6±0.6	3.2±0.7	2.8±0.8
	Stark Red gold	3.5±1.0	3.0±1.2	3.6±0.9	2.7±1.0	2.7±1.0	2.8±0.7
	Lidy Star	3.0±1.0	3.0±1.0	3.0±1.4	3.5±1.2	2.3±1.1	3.5±1.2
	Marilyn	1.2±0.4	3.1±1.2	2.6±1.2	2.4±0.6	2.9±0.7	2.1±0.6
	Zee Lady	2.8±0.9	3.2±0.7	2.9±0.6	3.1±0.7	2.6±0.5	3.0±0.7
C	O'Herny	3.4±0.8	3.7±0.9	3.4±0.5	2.9±0.8	2.7±1.1	3.3±0.5
	Tardi Belle	3.0±1.0	3.3±0.6	3.7±0.6	2.0±0.0	3.0±1.0	2.7±0.6
	Baby Gold 7	2.7±0.4	3.6±0.9	3.7±1.0	4.0±0.7	1.6±0.5	3.7±0.6

Intensity of aroma, freshness, sweetness and sourness scored from 1=less intense to 5=very intense; overall quality scored from 1= really poor to 5= excellent; color scored from 1= severe browning to 5= not browned. Mean values±standard deviation.

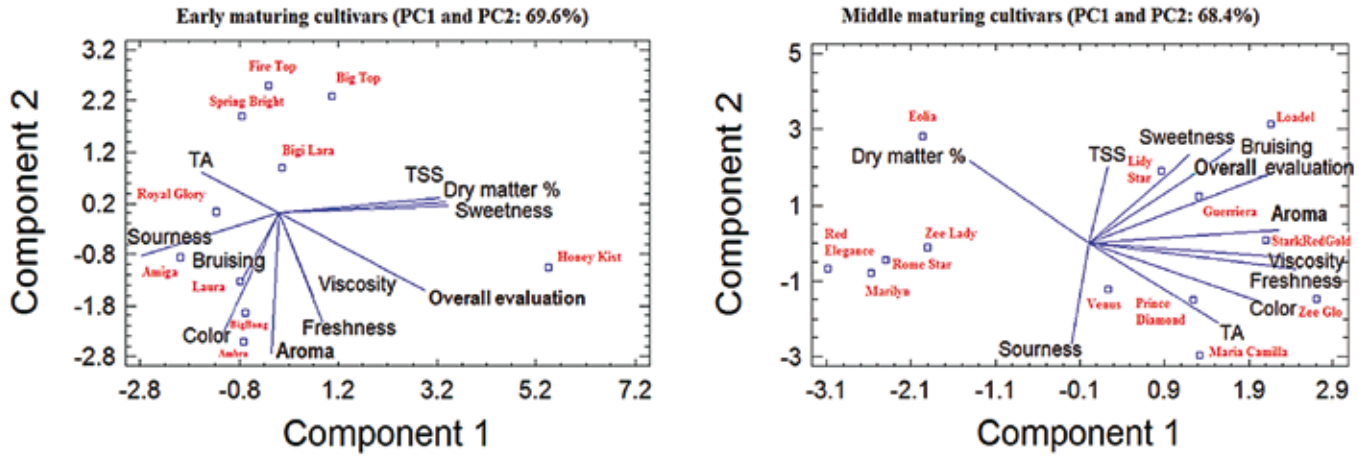


Fig. 1 - Biplot graphs for Group A (early maturing) and Group B (middle maturing) varieties. Grouping of peaches, nectarines and clingstone peaches according to their physical, chemical and sensorial attributes determined by PCA.

In the Early maturing cultivar biplot graph, the first group associates acidity and sourness, the second TSS, sweetness, dry matter and overall evaluation, and the third viscosity, color, bruising susceptibility, aroma, and freshness. Based on this positioning, opposite relationships between acidity and sensorial evaluation, and between sourness and sweetness were found. Specifically, PCA analysis in this study indicated that in Early maturing cultivars, 'Fire Top', 'Spring Bright', 'Royal Glory', 'Big Top' and 'Bigi Lara' were positioned in the upper quadrant along with acidity since TA values were higher than for other varieties. 'Honey Kist' was in the lower right quadrant along with TSS, sweetness, dry matter and overall evaluation. This positioning is in accordance with the chemical and sensorial analysis results; 'Honey Kist' showed the highest TSS value (16°Brix) associated with a high impact on consumer sweetness perception. Cultivars 'Laura', 'Ambra', 'Amiga', and 'Big Bang' were spread in the lower left quadrant according to sourness, color, bruising susceptibility, viscosity, and freshness; in particular 'Ambra' obtained a high sensorial score for aroma and color. It could be concluded that for Group A, nectarine 'Honey Kist' showed the highest TSS content, the lowest acidity, and an intermediate susceptibility to mechanical damage, with a potential positive impact on consumer preference together with 'Laura' and 'Ambra' that retained good color after processing.

In the Middle maturing cultivar biplot graph the first group was associated by TSS, sweetness, sensorial evaluation and bruising, the second by viscosity, freshness, color and TA, the third by sourness. Within this group, an opposite relationship between sweetness and sourness, and between dry matter content and TA was observed. 'Eolia' was in the left upper quadrant along with dry matter in accordance with chemical analysis. 'Lidy Star' and 'Guerriera' were in the upper right quadrant for sweetness and sensorial evaluation, 'Stark Red Gold' was associated with aroma due to the high overall score, while 'Loadel' differentiated for bruising due to the high incidence of mechanical damage. 'Zee Glo', 'Diamond Princess', 'Venus' and 'Maria Camilla' were spread around freshness, TA and color. 'Marilyn', 'Zee Lady', 'Rome Star' and 'Red Elegance' formed a third group positioned in the lower left quadrant between sourness and dry matter, due to their similar dry matter content (13%) and to the high score received for sourness. Within this group 'Stark Red Gold', 'Zee Glo', 'Venus', and 'Diamond Princess' resulted different from the others because of a higher overall evaluation and a lower susceptibility to bruising, indicating their suitability to minimal processing. On the contrary, clingstone peaches 'Loadel' and 'Eolia' were more susceptible to browning after blending, and although pleasant for sweetness and overall quality, their use for fresh convenience products would not lead to promising results.

As for Group C, the principal component analysis is not really meaningful due to the low number of cultivars. From the biplot graph (not shown) it was observed that 'Tardi Belle' was in the upper right quadrant associating with bruising susceptibility, due to the very high incidence to mechanical damage (100%). Although it was the most sensitive to mechanical damage, 'Tardi Belle' differentiated from the other varieties for the well balanced flavor. 'O'Henry' positioned in the lower right quadrant between TA and overall evaluation, while 'Baby Gold7' was in the left quadrant along with sweetness, aroma, freshness, and color, according to the sensorial results obtained.

In conclusion, this work confirms the extreme variability existing among genotypes in terms of sensorial quality, susceptibility to browning and to mechanical damage, and the importance of assessing screening to select the most suitable varieties for minimal processing. From these preliminary data, the best suited cultivars for minimal processing were 'Honey Kist', 'Laura' and 'Ambra' from Group A (early maturing), 'Stark Red Gold', 'Zee Glo', 'Venus' and 'Diamond Princess' from Group B (middle maturing); cultivars from Group C (late maturing) did not show many differences although this may be due to the limited number of varieties evaluated. Further studies are needed in order to better understand the biochemical and technological behavior and to extend the screening to other potentially interesting varieties.

References

AHVENAINEN R., 1996 - *New approaches in improving the shelf life of minimally processed fruit and vegetables.* - Trends in Food Science & Technology, 7: 179-187.

AMODIO M.L., COLELLI G., 2008 - *Effect of thermal treatment and dipping on quality and shelf-life of fresh-cut peaches.* - Adv. Hort. Sci., 22(1): 21-26.

BURDON J., MCLEOD D., LALLU N., GAMBLE J., PETLEY M., GUNSON A., 2004 - *Consumer evaluation of "Hayward" kiwifruit of different at harvest dry matter contents.* - Postharvest Biology and Technology, 34: 245-255.

BYRNE D., 2002 - *Peach breeding trends: a worldwide perspective*. - Acta Horticulturae, 592: 49-59.

BYRNE D.H., NIKOLIC A.N., BURNS E.E., 1991 - *Variability in sugars, acids, firmness, and colour characteristics of 12 peach genotypes*. - J. Am. Soc. Hort. Sci., 116(6): 1004-1006.

CABEZAS-SERRANO A.B., AMODIO M.L., CORNACCHIA R., RINALDI R., COLELLI G., 2009 a - *Suitability of five different potato cultivars (*Solanum tuberosum *L.) to be processed as fresh-cut products.* - Postharvest Biol. Technol., 53: 138-144.

CABEZAS-SERRANO A.B., AMODIO M.L., CORNACCHIA R., RINALDI R., COLELLI G., 2009 b - *Screening quality and browning susceptibility of 5 artichoke cultivars for fresh-cut processing.* - J. Sci. Food Agric., 89(15): 2588-2594.

CANDEL M.J.J.M., 2001 - *Consumers' convenience orientation towards meal preparation: conceptualization and measurement*. - Appetite, 36: 15-28.

CANTIN C.M., MORENO M.A., GOGORCENA Y., 2009 - *Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [*Prunus persica *(L.) Batsch] breeding progenies*. - J. of Agric. and Food Chemistry, 57: 4586-4592.

CASTELLARI L., MALAVOLTI A., COLOMBO R., RONDINELLI G.P., 2006 - *L'impiego dei "panel test" nella valutazione qualitativa di alcune nettarine emiliano-romagnole.* - Rivista di Frutticoltura, 7-8: 60-63.

CRISOSTO C.H., CRISOSTO G., NERI, F., 2006 - *Understanding tree fruit quality based on consumer acceptance.* - Acta Horticulturae, 712: 183-189.

CRISOSTO C.H., SCOTT JOHNSON R., DEJONG T., DAY K.R., 1997 - *Orchard factors affecting postharvest stone fruit quality.* - HortScience, 32(5): 820-823.

FOLEY D.M., DUFOUR A., RODRIGUEZ L., CAPORASO F., PRAKASH A., 2002 - *Reduction of* Escherichia coli *O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation.* - Radiation Physics and Chemistry, 63: 391-396.

GARCIA E.L., BARRETT D.M., 2002 - *Preservative treatments on fresh-cut fruits and vegetables*, pp. 276-303. - In: LAMIKANRA O. (ed.) *Fresh-cut fruits and vegetables*. Science, Technology and Market, CRC Press, Boca Raton, Florida, USA, pp. 467.

GIL M.I., TOMAS-BARBERAN F.A., HESS-PIERCE B., KADER A.A., 2002 - *Antioxidant capacities, phenolic compounds, and vitamin C contents of nectarine, peach and plumcultivars from California.* - Journal of Agriculture Food Chemistry, 50: 4976-4982.

HILAIRE C., MATHIEU V., 2004 - *Test hédoniques sur varieties de peche. D'abord, satisfaire le consommateur.* - Infos-Ctifl, 162: 32-35.

IGLESIAS I., ECHEVERRÍA G., 2009 - *Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance*. - Scientia Horticulturae, 120: 41-50.

LIVERANI A., GIOVANINI D., BRANDI F., MERLI M., 2003 - *Le pesche subacide.* - L'Informatore Agrario, 31: 43-49.

MITCHELL G.F., KADER A.A.,1989 - *Factor Affecting deterioration rate in peaches, plums and nectarines - growing and handling for fresh market.* - Division of Agriculture and Natural Resources, University of California, Publication no. 3331.

NICOTRA A., CONTE L., 2003 - *Nuove Tipologie di frutto per il mercato delle pesche: nascono la serie "Ufo" e "Ghiaccio"*. - Rivista di Frutticoltura, 7-8; 20-25.

Adv. Hort. Sci., 2012 26(1): 39-43

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

A. Luvisi*, A. Panattoni*, R. Bandinelli, E. Rinaldelli***, M. Pagano***, E. Triolo***

** Dipartimento di Coltivazione e Difesa Specie Legnose, Sezione di Patologia Vegetale, Università degli Studi di Pisa, Via Del Borghetto, 80, 56124 Pisa, Italy.*

*** Associazione Toscana Costitutori Viticoli, TOS.CO.VIT, Via Vecchia di Marina, 6, 56010 San Piero a Grado (PI), Italy.*

**** Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Sezione di Coltivazioni Arboree, Università degli Studi di Firenze, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.*

Key words: conservative breeder, foundation block, electronic identification.

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

Received for publication 2 February 2012
Accepted for publication 5 March 2012

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-

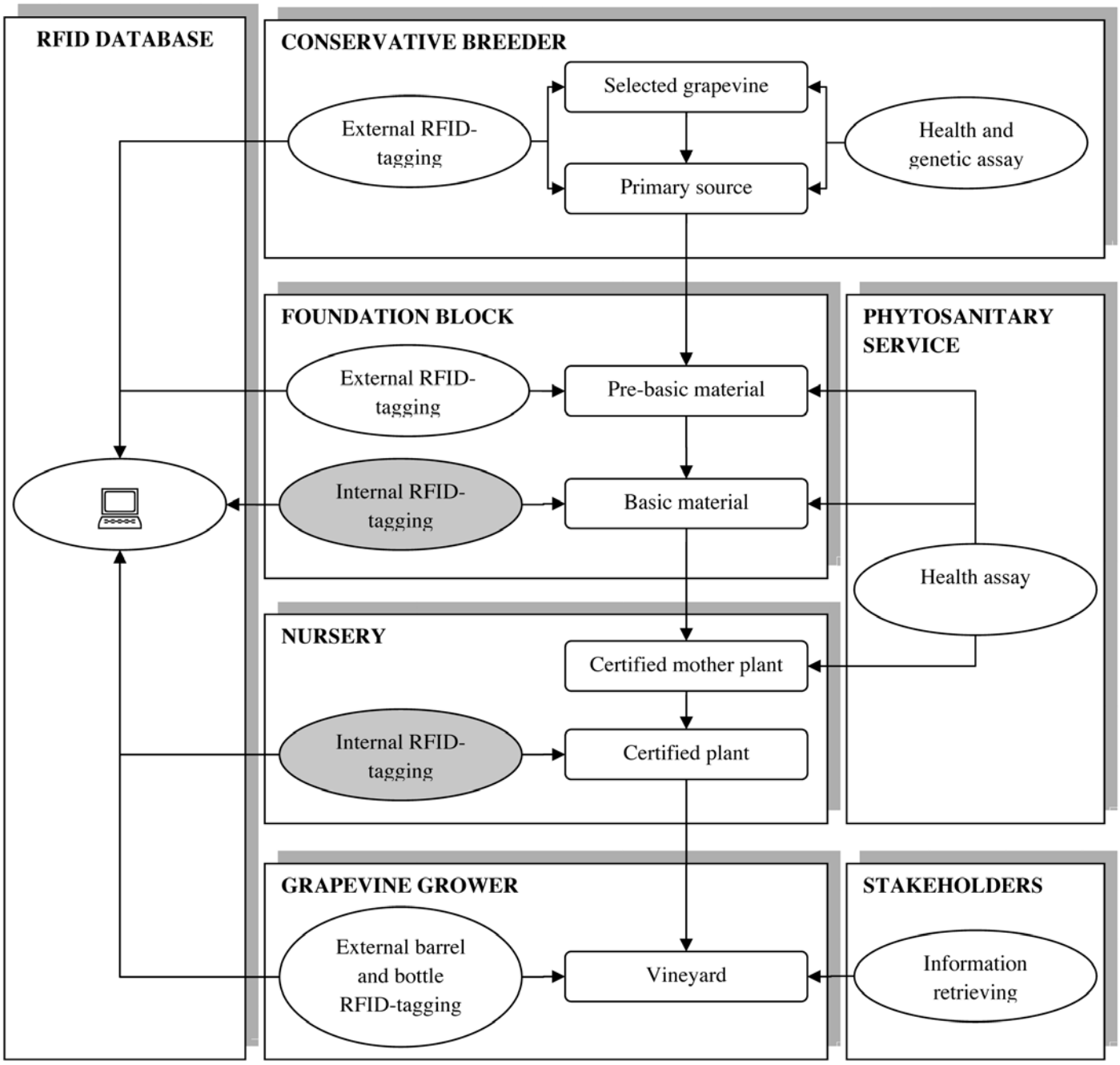


Fig. 1 - Data flow within RFID-tagged grapevine production chain.

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.

References

BANDINELLI R., TRIOLO E., LUVISI A., PAGANO M., GINI B., RINALDELLI E., 2009 - *Employment of radiofrequency technology (RFId) in grapevine nursery traceability*. - Adv. Hort. Sci., 23(2): 75-80.

CERRI S., PANATTONI A., TRIOLO E. 1993 - *Studio, progetto e sperimentazione di una procedura semiautomatica per l'analisi di alterazioni del legno in barbatelle di vite*. - Consiglio Nazionale Ricerche, Nota Interna, B4-25: 1-24.

COLLINS J., 2005 - *Wine bottles get corked with RFID*. - http://www.rfidjournal.com/article/articleview/2117/1/1/

EUROPEAN COMMISSION, 2011 - *Electronic identification of bovines to further strengthen food safety and animal health in the EU*. - http://europa.eu/rapid/pressReleasesAction.do?reference=IP/11/991&format=HTML&aged=0&language=EN

LAUNOIS A., 2008 - *RFID tracking system stores wine bottle data*. - http://www.foodproductiondaily.com/Packaging/RFID-tracking-system-stores-wine-bottle-data

LUVISI A., PAGANO M., BANDINELLI R., RINALDELLI E., GINI B., SCARTÒN M., MANZONI G., TRIOLO E., 2011 - *Virtual vineyard for grapevine management purposes: a RFID/GPS application* - Computers and Electronics in Agriculture, 75: 368-371.

LUVISI A., PANATTONI A., BANDINELLI R., RINALDELLI E., PAGANO M., GINI B., TRIOLO E., 2010 b - *RFID microchip internal implants: effects on grapevine histology*. - Scientia Horticulturae, 124: 349-353.

LUVISI A., TRIOLO E., RINALDELLI E., BANDINELLI R., PAGANO M., GINI B., 2010 a - *Radiofrequency applications in grapevine: From vineyard to web*. - Computers and Electronics in Agriculture, 70: 256-259.

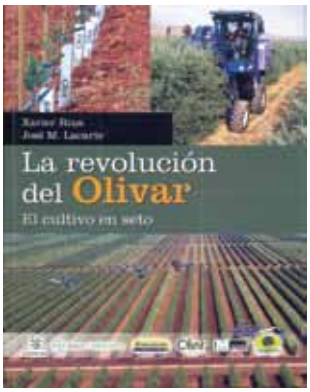
PAGANO M., BANDINELLI R., RINALDELLI E., PANATTONI A., TRIOLO E., LUVISI A., 2010 - *RFID technology for clonal selection purposes*. - Adv. Hort. Sci., 24(4): 282-284.

PORTO S.M.C., ARCIDIACONO C., CASCONE G., 2011 - *Developing integrated computer-based information systems for certified plant traceability: Case study of Italian citrus-plant nursery chain*. - Biosystem Engineering, 109(2): 120-129.

SØRENSEN C.G., FOUNTAS S., NASH E., PESONEN L., BOCHTIS D., PEDERSEN S.M., BASSO B., BLACKMORE S.B., 2010 - *Conceptual model of a future farm management information system*. - Computers and Electronics in Agriculture, 72: 37-47.

SØRENSEN C.G., PESONEN L., BOCHTIS D.D., VOUGIOUKAS S.G., SUOMI P., 2011 - *Functional requirements for a future farm management information system*. - Computers and Electronics in Agriculture, 76: 266-276.

BOOK REVIEWS



LA REVOLUCIÓN DEL OLIVAR. EL CULTIVO EN SETO. *Rius Xavier*, and *José M. Lacarte*. Agromillora Iberia, Subirata, Barcelona, Spain, 2010, pp. 340. ISBN 978-0-646-53737-5.

The need to lower costs to manage olive orchards in order to make the production of extra virgin olive oil more profitable has been for some time an important objective for olive growers worldwide. In this regards, mechanization has helped: today it is at its best when it comes to pruning and harvesting. In this context, the volume by Xavier Rius and José M. Lacarte is timely and offers a wealth of information. The title itself suggests the quality of the content: research and experimentation that have radically transformed an obsolete management system into new hope for olive growing in the third millennium.

The text is divided into four chapters: 1. *El aceite de oliva en el mundo* (Olive oil in the world); 2. *La oportunidad de negocio en las plantaciones de olivar superintensivo* (The economic opportunity in the superintensive planting system olive groves). 3. El cultivo superintensivo del olivar: respuesta a las necesidades del sector (The superintensive planting system in olive groves: response to sector needs). 4. *El cultivo superintensivo en los principales países oleícolas (España, Italia, Grecia, Portugal, Túnez, Marruecos, Estados Unidos, Chile, Australia, Argentina, resto del mundo)* (The superintensive planting system in the major olive-producing countries: Spain, Italy, Greece, Portugal, Tunisia, Morocco, USA, Chile, Australia, Argentina, Rest of the World).

A wealth of tables, figures and color image aid consultation of the text and add value to its presentation.

Considering the global interest of the topic, translation of the text into English is desiderable and perhaps predictable in the near future.

Enrico Rinaldelli

IL PAESAGGIO MANTOVANO. NELLE TRACCE MATERIALI, NELLE LETTERE E NELLE ARTI. IV. IL PAESAGGIO MANTOVANO DALL'ETÀ DELLE RIFORME ALL'UNITÀ (1700-1865).
Atti del Convegno di Studi (Mantova, 19-20 Maggio 2005)(The Mantuan landscape. From material, literary and artistic traces. The Mantuan landscape from the period of Reform to Unity (1700-1865). Prooceedings from the study convenction in Mantua, 19-20 May 2005). *Camerlenghi E., V. Rebonato,* and *S. Tammaccaro* (eds.). Leo S. Olschki, Florence (Italy), 2010. pp. x + 454 + 7 figures and 70 plates, 63 of which in color. ISBN 978-88-222-5845-8. € 55.00.

The Proceedings, edited by E. Camerlenghi, V. Rebonato, and S. Tammaccaro, of the study convenction held in Mantua, 19-20 may 2005 have recently been published. They are the fourth volume of the work on the Mantuan Lanscape presented by *Accademia Nazionale Virgiliana di Scienze, Lettere, e Arti* and edit by Leo S. Olschki.

The various contributions which make up the volume, and include images from the period, have been prepared by outstanding experts in the field of historical Italian landscapes and offer stimulation for debate about the causes behind the development of the Mantua area during the subject period. Ample space is dedicated to hydraulic conditions, which were tied to the control of power of Padanian principalities, agronomic aspects and the evolution of crops, as well as to literary sources, from Folengo to Renaissance novelists. All this, without overlooking the urban landscape, which in this historic period in Mantua offered moments of great splendor.

Some of the chapters clearly highlight the changes which occurred in the landscape, above all with regard to the management of water resources, while others focus on the parks, gardens and urban green spaces of Mantua during the period.

The text, which includes a generous list of bibliographic references, also contains illustrations (both color and black and white) and color photos. This volume is a valuable addition, under a scientific and technical profile as well as from a historical and cultural point of view, to the vast literature that exists on the evolution of historical-landscape areas. The work thus offers the reader insight and knowledge about numerous and important aspects of the landscape which make up our image of the Mantuan territory.

Francesco Ferrini

L'ARCHITETTO SAPIENTE. GIARDINO, TEATRO, CITTÀ COME SCHEMI MNEMONICI TRA IL XVI E IL XVII SECOLO. *Koji Kuwakino.* Giardini e paesaggio, vol. 28. Leo S. Olschki, Florence (Italy), 2011. pp. xxiv-326 + 70 figures and 7 plates in colour. ISBN 978-88-222-6046-8. € 29.00.

In this work, by the Japanese scholar Koji Kuwakino and published by Leo S. Olschki of Florence, the little-known relationship between architecture, the art of memory and encyclopaedism of the first modern age is examined through examples of the fruitful interaction between words, images and space. The subtitle of the book underlines the investigated subject: garden, theater, and city as mnemonic schemes in the 16th and 17th centuries. This architectonic typology offers the schemes of a complex conceptual construction able to collect, order and internalize the enormous quantity of knowledge acquired and accumulated over the centuries, from the ancient world up to the contemporary age. The author analyzes the ideal gardens of Agostino del Riccio and Giovan Battista Ferrari, the universal theater of Samuel von Quiccheberg and the mnemonic city of Cosma Rosselli. As the author points out in the introduction, it is "an ideal journey" aimed at analyzing "three architectonic typologies often utilized as mental spaces: the garden, the theater, and the city". These spaces "are not so much concretely real, but rather are ideal plans created with words, and the authors are not professional architects but gifted men and *virtuosi* who understand architecture". Dr. Kuwakino goes on to explain that these examples of spaces "set outside the specialized field of 'ars aedificatoria', present only in text and for the most part unknown to historians, reveal, as in filigree, aspects that were previously hidden or scarcely examined despite being of considerable interest and rich in suggestion". The analysis presented in the book aims to offer a complementary reading and enrichment of the history of 16th century architecture.

Through this work the author succeeds in shining light on fundamental cognitive aspects of the architectonic culture of the first modernity which previously had not been sufficiently emphasized. "*L'architetto sapiente*" demonstrates clearly how much architecture is under the influence of the spirit of the times - architectonic space become, for example, a metaphor for knowledge - and how much architecture has contributed to the building of new structures of modern thought.

After the author's introduction, the text is dived into six chapters: "*L'arte della memoria architettonica*" (The art of architectonic memory); "*Il giardino del tardo cinquecento come luogo del pensiero*" (The garden in the late 16th century as a place for thought); "*La rappresentazione emblematica del sapere enciclopedico nel giardino mnemonico di Del Riccio*" (Emblematic representation of encyclopaedic knowledge in the mnemonic garden of Del Riccio); "*Il 'Paradiso Celeste' nel giardino italiano*" (The 'Paradiso Celeste' in the italian garden); "*Il grande teatro del pensiero creativo*" (The great theater of creative thought); and "*Città celeste e 'loca communia'*", (The celestial city and the 'loca communia'), followed by the conclusions. The text includes a ample list of bibliographic references and is enriched by various illustrations and photos (black and white and color) which highlight the presented material.

This work is clearly a precious addition to the existing literature on landscape studies, from both scientific and historical-cultural points of view, and offers, page after page, new and significant knowledge to the reader.

Cinzia Silori

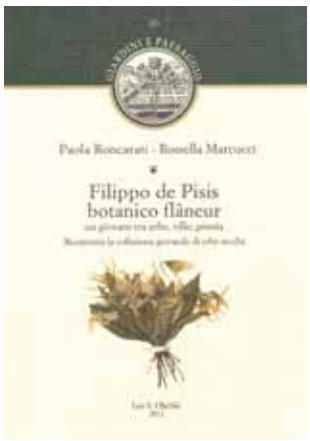
TERRITORI DELLE ACQUE. ESPERIENZE E TEORIE IN ITALIA E IN INGHILTERRA NELL'OTTOCENTO (TERRITORIES OF WATER: EXPERIENCES AND THEORIES IN ITALY AND ENGLAND IN THE 19TH CENTURY). Aquae Studi e testi sulle terme, 4. *Gabriele Corsani* (ed.). Leo S. Olschki, Florence (Italy), 2010. pp. 164. ISBN 978-88-222-5988-2. € 20.00.

Water, promethean and domestic, represents the magnificent destiny of progress as the most radical adherence to the spirit and laws of nature.

Certainly, it is not easy to reduce down to a single text the myriad aspects of water and its interaction between the earth and the living things which occupy our planet. For example, water as power and energy but also as nourishment for life and the human spirit. In this context, Gabriele Corsani has deftly succeeded in telling the story of water in Italy and England over the course of the 19th century. The volume, which contains a wealth of bibliographic information and illustrations from the period, is a complete work from different points of view: an interesting historical analysis and a precious bibliographic source for historians and researchers.

The text, compiled by notable authors, includes the following ten contributions: Gabriele Corsani, *Territori delle acque: esperienze e teorie in Italia e in Inghilterra nell'Ottocento* (Territories of water: experiences and theories in Italy and England in the 19th century). Antonello Boatti, *Storici e scrittori lombardi dell'Ottocento di fronte al paesaggio. Corsi d'acqua naturali e artificiali tra agricoltura e industria negli scenari disegnati da Carlo Cattaneo e Cesare Cantù* (Lombard historians and writers in the 19th century in a landscape context. Natural and artificial waterways in agriculture and industry in scenes drawn by Carlo Cattaneo and Cesare Cantù. Carlo Cattaneo, *Prospetto della navigazione interna delle province lombarde con alcune notizie sulla loro irrigazione ("Il Politecnico", 1841)* (Prospectives of navigation within the province of Lombardy and notes on irrigation) *("Il Politecnico", 1841)*. Cesare Cantù, *Storia di Milano, Diocesi e Provincia di Milano, cap. V. Acque* (History of Milan, Diocese and Province of Milan, Chapter V. Water). Marco Geddes da Filicaia, *Corrado Tommasi-Crudeli e il tema dell'acqua ai tempi del colera* (Corrado Tommasi-Crudeli and the topic of water during times of cholera). Corrado Tommasi-Crudeli, *La canalizzazione delle città* (Canalization of the city). Katia Caldari, *Alfred Marshall e l'importanza delle risorse naturali* (Alfred Marshall and the importance of natural resources). Alfred Marshall, *L'acqua come un elemento della ricchezza nazionale* (Water as an element of national wealth). Gabriele Corsani, *La Appendix howardiana: tecnica e retorica comunitaria per le acque ludiche di città giardino* (The Howardian appendix: technique and community rhetoric for water in the city garden). Ebenezer Howard, *Appendice. L'approvvigionamento idrico* (Appendix. The provisioning of water).

Enrico Rinaldelli



FILIPPO DE PISIS BOTANICO FLÂNEUR, Un giovane tra erbe, ville, poesia. Ricostruita la collezione giovanile di erbe secche. Roncarati Paola and Rossella Marcucci. Giardini e paesaggio, vol. 30. Leo S. Olschki, Florence (Italy), 2011. pp. 208. ISBN 978-88-222-6139-7. € 28.00.

This attractive volume by Paola Roncarati and Rossella Marcucci presents ample and detailed documentation about an interesting but rarely mentioned, multifaceted man: Filippo De Pisis. De Pisis is identified principally as a notable painter of the early 20th century but he was also, from a young age, a talented writer and poet as well as a passionate entomologist and botanist. Indeed, it is on these latter aspects of his career that the two authors, through a combination of their areas of expertise, have focused the current work, offering an important contribution to the complex and heterogeneous biography of the painter.

The volume begins with an introduction by Lucia Tongiorni Tomasi and Luigi Zangheri entitled "L'erbario essiccato, un inedito tributo alla personalità di Filippo De Pisis", (The dried herbarium, a tribute to Filippo De Pisis), which is then followed by a carefully written preface by Gianni Venturi, who admirably comments on the meaning and significance of the work: "It is clear that this book is difficult to define as a study of an herbalist, just as every work of poetry includes a multitude of meanings which render it precious, not only for a specific interest, but above all as an important step toward understanding the life and works of the artist in greater depth."

The text is divided into two parts. The first is dedicated to "Le implicazioni culturali di una passione botanica" (The cultural implications of a passion for botany) and the second to "Luoghi di erborizzazione, escursioni della mente, passeggiate dello sguardo" (Places for herbalism, excursions of the mind, and a stroll for the eyes). Finally, there is an analytical comment by the authors on the Estrosità nell'erbario: lo sguardo "asistematico" di un botanico originale". (Creativity in the herbarium: an asystematic outlook by an original botanist). The work also includes a series of documents, including an article by Giuseppe Viviani "A passeggio con De Pisis" (A walk with De Pisis) and a rich bibliography,.

Enrico Rinaldelli