Journal of Applied Horticulture

ISSN 0972-1045
Vol. 12, No. 2, July-December, 2010

Journal of
THE SOCIETY FOR ADVANCEMENT OF HORTICULTURE

JOURNAL OF APPLIED HORTICULTURE
Vol. 12, No. 2, July-December, 2010

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Journal of Applied Horticulture, 12(2): 87-92, July-December, 2010

Effects of water inclusion in microclimate modification systems for warm and cool season vegetable crops on temperature and yield

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Abstract

Four microclimate modification methods including spun-bonded and slitted low tunnels both with and without the addition of water-filled plastic tubes were tested for their effect on early and total yields of warm and cool season vegetable crops in Morgantown, West Virginia, USA. Peppers, tomatoes, radishes, and lettuce were organically grown in 2006 and 2007. Early season pepper yields were higher when water tubes were included with low tunnels while early tomato yields did not differ. Total yields for warm season crops in some microclimate modification treatments were higher than the control, and harvests started up to four weeks earlier in the spring. Cool season crop yields in the four treatments showed no increase over the control despite one to three weeks earlier harvests for radish and lettuce. These results show potential for earlier safe planting dates and increased yield, especially in warm season vegetable crops using low tunnels and water tubes. Additionally, economic analysis demonstrated a potential for increased profits over control plots using these microclimate modification techniques.

Key words: Slitted polyethylene low tunnel, spun-bonded row cover, water tubes, organic vegetable production, tomato (*Solanum lycopersicon*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), bell pepper (*Capsicum annuum*)

Introduction

Techniques to modify the microclimate have been used for many years by growers on a variety of scales and climates (Wells and Loy, 1985; Wittwer and Castilla, 1995). Microclimate modification can extend growing seasons by allowing both earlier spring harvests and later fall crops (Lamont, 2005; UK, 2007) while producing several main benefits for growers. An increase in vegetable production is possible because of a lengthened growing season in addition to a concomitant increase in earnings because of extended sales and price premiums for early and late season produce. There is also a potential benefit of an expanded customer base because produce can be made available for a greater portion of the year (Bachmann, 2005; UK, 2007). Advances in crop production techniques and technologies now allow farmers greater levels of control over crop growing conditions. These technologies include structures, such as low and high tunnels, employed to create specific microclimates around developing crops (Lamont, 2005; Wells and Loy, 1985; Wittwer and Castilla, 1995). However, initial costs of these techniques vary and some growers may desire the benefits of microclimate modification while using lower cost techniques, such as spun-bonded, slitted, or perforated row cover materials applied as low tunnels instead of high tunnels or greenhouses.

Row covers and low tunnels, often used with plastic mulches, have been shown useful in many climates for a number of crops. The purpose of such covers is to modify temperatures in a way that has the potential to alter the growth and yield of horticultural crops (Wells and Loy, 1985). Muskmelons (*Cucumis melo*) have been studied often in row cover and low tunnel research with several reports of increased early and total yields (Hemphill Jr. and Mansour, 1986; Ibarra *et al.*, 2001; Jenni *et al.*, 1998; Loy and Wells, 1982; Taber, 1993). In addition, spun-bonded row covers have also been shown to enhance early season tomato yields (*Solanum lycopersicon*) (Reiners and Nitzsche, 1993) and raise watermelon (*Citrullus lanatus)* yields by increasing transplant survival and earlier harvests (Marr *et al.*, 1991). The combination of row covers and mulches has also shown increased growth and yields in watermelon (Soltani *et al.*, 1995) and cucumber (*Cucumis sativus*) (Nair and Ngouajio, 2010).

Row covers and low tunnels can also be beneficial in the production of cool season crops. Floating row covers and/or tunnels have been shown to allow earlier planting and harvest of broccoli (*Brassica oleracea*) (Westcott *et al.*, 1991) and increased maturity and yield of crisphead lettuce (*Lactuca sativa*) (Rekika *et al.*, 2009). Chinese cabbage (*Brassica rapa*) crops grown under row covers and low tunnels also showed higher yields than uncovered plots due to increased air and soil temperatures (Moreno *et al.*, 2002). Other benefits, such as potential for reduced disease pressure and increased marketable yield, have also been observed with row cover use in bell pepper (*Capsicum annuum*) and watermelon production (Alexander and Clough, 1998; Avilla *et al.*, 1997; Walters, 2003).

Additionally, row covers and low tunnels have the potential to be combined with other microclimate modifying techniques. The use of water-filled tubes has been tested in growing systems for its ability to moderate both high and low ambient temperatures and protect temperature sensitive crops (Aziz *et al.*, 2001; Jenni *et al.*, 1998). Jenni *et al.* (1998) reported that certain combinations of tunnels, mulches, and thermal water tubes in the growing environment could lower chilling injury and increase muskmelon

early yields. Additional research (Aziz *et al.*, 2001) on the use of water tubes in vented low tunnels showed reduced temperature fluctuations which had the potential to indirectly affect plant growth rates.

These relatively low-cost techniques, such as low tunnels and water tubes, can potentially increase the cropping season by allowing earlier planting and harvesting and protecting warm season crops during low-temperature events early in the season. Such techniques have been used on a variety of crops with research often focusing on tomatoes and melons. The effect of these technologies has not been as extensively investigated on some cool season crops. Additionally, the inclusion of water tubes in microclimate modification systems has been tested on a relatively narrow range of crops and to an even lesser extent to study planting crops earlier despite the real possibility of doing so. In most research, controls are planted at the same time as the microclimate modification techniques are applied, ignoring the practical aspect of being able to plant earlier with the additional protection afforded by microclimate modification.

The purpose of this study was to compare the effects of low tunnels and the inclusion of water tubes on air and soil temperatures and total and early yield of warm season pepper and tomato crops as well as cool season lettuce and radish crops. In addition, an economic analysis was carried out to determine the feasibility and profitability of the microclimate modification methods under investigation. These techniques could be used as a cost-effective way for growers to enhance early yield through modifying growing environments without investment in higher input methods, such as high tunnels or greenhouses.

Materials and methods

Location and plant material: The West Virginia University Plant and Soil Sciences Organic Farm in Morgantown, West Virginia, under certified organic production since 2003, was the location for our experiments. The soil was a moderately well-drained silt loam classified as a fine-silty, mixed, semiactive, mesic Typic Fragiudults.

Organic seed was obtained from Johnny's Selected Seeds (Winslow, ME) and High Mowing Seeds (Wolcott, VT), and all crops were grown under USDA National Organic Program rules. The warm season crops were tomato ('WV '63') and bell pepper ('Orion'). Cool season crops included lettuce ('Parris Island Cos') and radishes (*Raphanus sativus*) ('Easter Egg' and 'Pink Beauty'). The tomato seeds used in this experiment were collected from the previous growing season at the WVU Organic Research Farm. Tomato, pepper, and lettuce plants were sown in the West Virginia University Plant and Soil Sciences greenhouse and later transplanted to the field while radishes were direct seeded. Tomato and pepper plants were sown eight weeks before planting, and were transplanted to cell packs four week after seeding. Lettuce was planted in the field directly from the seeding flats (288-cell) approximately four weeks after seeding. The greenhouse medium consisted of 50% composted dairy manure (WVU Animal Sciences Farm, Morgantown, WV), 25% peat moss (BFG Horticultural Supply, Burton, OH), and 25% perlite (BFG Horticultural Supply, Burton, OH).

Experimental treatments: The experiment was conducted in both the 2006 and 2007 growing seasons. The four low tunnel treatments and the control were arranged in a randomized complete block design with three replications. The dimensions of the fifteen plots were 3.7 x 4.9 m with a total area of 18.1 m^2 per plot. The four crops in each replicate were planted side-by-side in four 4.9 m long rows each planted to one crop with 0.75 m between-row spacing. The in-row spacing for tomatoes (6 plants per replicate) was 0.75 and 0.5 m for peppers (8 plants per replicate). Lettuce (12 plants per replicate) was spaced at 0.3 m in-row while radish seeds were sown at approximately 100 seeds m^{-1}.

The control consisted of the four crops grown without the use of low tunnels or water tubes described below. The first treatment consisted of spun-bonded polypropylene (Agribon 17 g m^{-2}, Hummert International, Earth City, MO) used as a floating cover for cool season crops and supported above the warm season crop by 3.4 mm wire hoops to form low tunnels. All coverings were secured by placing soil on the edges. The second treatment used the same spun-bonded covering but 15 cm-diameter water-filled polyethylene (6 mil) tubes (U-Line Shipping Supply, Chicago, IL) were placed on the soil on either side of the crop rows in 1 m sections heat sealed on each end after filling. In each replicate, approximately 100 L of water was contained in the tubes. The third treatment used slitted polyethylene (0.5 mil) stretched over hoops to form low tunnels (Hummert International, Earth City, MO) as described above. The fourth treatment consisted of the slitted polyethylene low tunnel with the addition of water tubes as described in the second treatment.

The control plots were established according to predicted frost-free dates using long-term temperature data for the area (National Climactic Data Center). Warm season crops were planted when only a 10% chance of frost remained while cool season crops were planted when temperatures were expected to reach levels necessary for germination and/or growth. Planting dates in the experimental treatments were calculated combining predicted low temperatures, as with the control, with the addition of temperature protection provided by the low tunnel methods, as listed by the manufacturer and based on our own experience. The spun-bonded and slitted low tunnel treatments were therefore assumed to provide approximately 1.1 to 2.2 °C and 0.6 to 1.7 °C of frost protection, respectively. This temperature protection resulted in earlier planting for the experimental treatments because an earlier date was associated with a 10% chance of frost. The year-to-year variations in planting dates (Table 1) occurred because of site-specific weather and soil moisture conditions.

The control and all treatment plots were fertilized through the addition of composted dairy manure (WVU Animal Sciences Farm, Morgantown, WV) at a rate of 55 kg $plot^{-1}$ (18.1 m^2) prior to spring planting in both years. Yearly soil testing confirmed adequate soil nutrient status to support vegetable crop growth with these levels of compost addition (data not shown). Tomatoes and bell peppers were mulched at planting with a double layer of newspaper under 5 cm of hay while lettuce and radish were transplanted or seeded directly in the soil without mulching. The low tunnels were installed at planting for all crops in both 2006 and 2007. They were kept in place until harvest for cool season crops and removed around 10 June in both years for the warm season crops. This was done to avoid excessive heat buildup under the covers (Decoteau, 2000).

Table 1. Planting and harvest dates for cool and warm season crops across the two years of the experiment

Date	Season	2006		2007	
		Control	Treatments	Control	Treatments
Plant date	Cool Season	19 Apr.	31 Mar.	30 Apr.	9 Apr.
	Warm Season	24 May	5 May	27 May	9 May
Harvest date	Cool Season	9 June	15 May	8 June	24 May
	Warm Season	Through 25 Aug.	Through 25 Aug.	Through 4 Sept.	Through 4 Sept.

Data collection*:* Both air (2.5 cm above soil line to capture possible water tube effects on air temperature) and soil (10 cm depth) temperature data were collected hourly in one plot of each treatment and the control with data loggers (Spectrum Tech., East-Plainfield, Il) protected from solar radiation by white plastic tubes. Photosynthetically active radiation (PAR) light measurements at plant canopy height were gathered using a quantum light sensor (Spectrum Tech., East-Plainfield, Il) logging hourly in a control plot with comparison light levels in the experimental plots taken in two-week intervals in a rotation through the season. Yield data were gathered on all three replicates. Early yields were not obtained for cool season crops as a single once-over harvest was conducted when the plants reached marketable maturity. Tomato and pepper fruit were harvested weekly on all plots at marketable maturity and all fruit harvested prior to the appearance of ripe fruit in the control was described as early yield.

Labour and input costs were also gathered for each experimental treatment. To estimate input costs, time and materials needed for each experimental treatment (in excess of those needed for the control) were calculated on a plot basis (18.1 m^2). Additional labour needed to initiate the microclimate modification treatments was calculated at \$6.00 hr^{-1}, while the low tunnel and water tube materials were calculated using the purchase price of the spun-bonded and slitted coverings, and water tubes needed for each individual plot. To determine potential returns in each plot, the treatment yield averages for all four crops were multiplied by actual farmer's market sales prices for that crop and combined to estimate the total plot value. In the warm season crops, early season yields (those harvested before the first ripe fruit in the control) were valued higher than later yields due to price premiums in the market where this produce was sold. The additional labor and input costs of the four experimental treatments were then subtracted from the potential returns calculated from both early and later season yields times the sales price to obtain a measure of how potentially profitable each treatment was in each year of the experiment. The formula for these calculations is then: Net returns =((early season average yields x early season price) + (regular season average yields x regular season price)) – (labour + material inputs).

Statistical analysis: Yield data from the 2006 and 2007 seasons were combined into a single statistical analysis because no significant treatment-by-year interactions were present. The General Linear Model procedure was used to analyze yield data with LSDs to show treatment differences at $\alpha = 0.05$ using SAS version 9.1 (Cary, NC). Contrasts were used to compare control vs. others, spun-bonded vs. slitted low tunnel treatments and treatments with vs. without water-filled tubes.

Results and discussion

Air and soil temperatures: In the spring seasons of 2006 and 2007, observed air temperatures were generally higher by 1 to 3 ºC in slitted low tunnels and 2 to 3 ºC under spun-bonded low tunnels (Fig. 1). Although statistical analysis was not carried out on the collected temperature data in this experiment, trends in air temperature correspond with other published work. Increases in air temperature and heat units in low tunnels and row cover treatments over bare ground or mulch have been often reported in previous research (Motsenbocker and Bonnano, 1989; Ibarra *et al.*, 2001; Rekika *et al.*, 2009; Waterer, 2003; Loy and Wells, 1982). Waterer (1993) showed spun-bonded and perforated polyethylene tunnel increased mean daily air temperature 3 to 6ºC, while Moreno *et al.* (2002) reported 5 to 7ºC increases. Jenni *et al.* (1998) reported spun-bonded and polyethylene tunnel air temperature increases of around 2 to 3ºC and 3 to 5ºC, respectively. Aziz *et al.* (2001) also consistently showed an average air temperature increase in perforated low tunnels with water tubes over uncovered control plots. Additionally, water tubes included in tunnels were shown to increase minimum and decrease maximum air temperatures over treatments with tunnels but no thermal water tubes (Aziz *et al.*, 2001).

Fewer consistent microclimate modification impacts on soil temperature were observed in this study (Fig. 1) than have been reported previously. Soltani *et al.* (1995) reported mean soil temperatures of 1 to 3 ºC higher with spun-bonded row covers and polyethylene low tunnels over mulch, while Hemphill, Jr. and Mansour (1986) and Moreno *et al.* (2002) reported 2 to 4 ºC and 5 ºC increases, respectively. Jenni *et al.* (1998) demonstrated that perforated low tunnel increased soil temperatures by 1 to 3 ºC

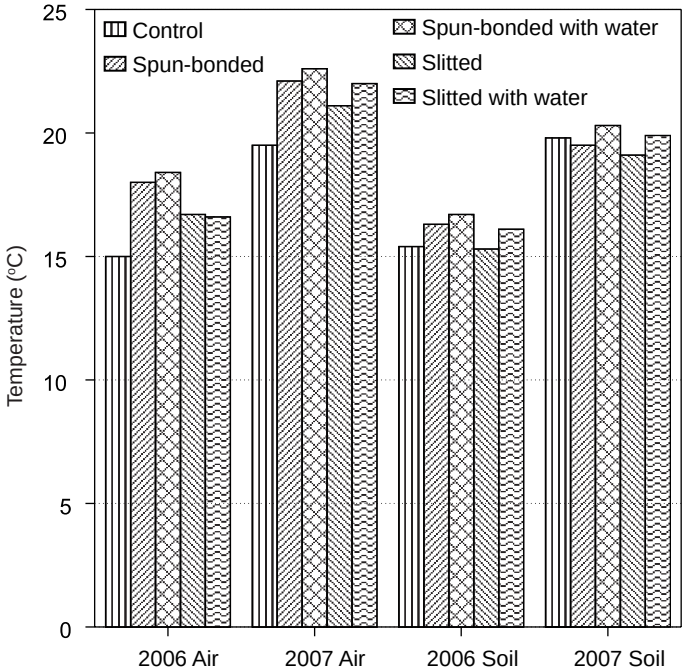

Fig. 1. Mean air and soil temperatures for the spring 2006 (4/4 - 6/13, N=71 days) and 2007 (5/1 –6/11, N=42 days) seasons. Each treatment mean represents the temperatures recorded while microclimate modification techniques were employed.

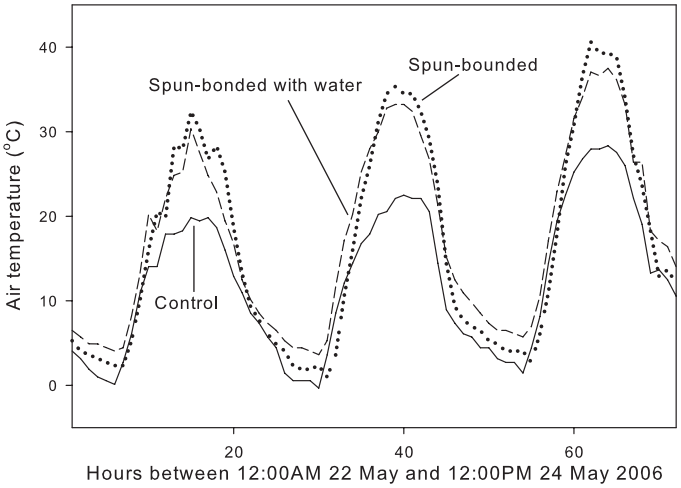

Fig. 2. Hourly air temperatures experienced from 22 May 2006 through 24 May 2006 in the control, spun-bonded and spun-bonded with water treatments. Impacts of the microclimate modification treatments on nighttime low and daytime high temperatures are illustrated.

while water tubes under non-perforated low tunnels increased soil temperatures by 2 to 5 ºC over mulch treatments alone. A possible explanation for our inconsistent trends in soil data as compared to other research is that many other reported soil temperatures differences were measured when low tunnels were used over plastic mulch. Plastic mulches could have produced greater differences than the organic hay mulches used in our work in addition to differences in depths of soil temperature measurement. Differences in material thickness of plastic present further difficulties in accurately comparing air and soil temperatures of spun-bonded and slitted or perforated polyethylene covers with previous research.

While not illustrated by average temperatures over the course of our experiment, experimental microclimate modification treatments demonstrated the potential impact of the low tunnel and low tunnel with water tube microclimate modification methods on temperature. Trends toward increased minimum nighttime air temperature in the spun-bonded tunnels versus the control are depicted. Impacts of water tubes on daytime air temperature were more variable (Figs. 2 and 3). This possible impact on minimum nighttime air temperatures, observed primarily in spun-bonded tunnels, could have contributed to plant survival in low temperature events prior to the planting of the control warm season crops. In 2006, between the experimental treatment planting date of 5 May and the control planting date of 27 May, low temperature events occurred on 22 May through 24 May (Fig. 2), but plant damage due to these low temperatures was not observed.

Photosynthetically active radiation (PAR): Two-week PAR averages for slitted polyethylene and spun-bonded treatments were measured as a percentage of the control for the same two-week intervals of the 2007 growing season. The slitted low tunnel received an average 85% of the PAR of the control, while the spun-bonded tunnels averaged 74% of the control PAR values (data not shown). These values are consistent with published PAR levels in season extension systems. Soltani *et al.* (1995) described 70 to 80% transmittance levels for spun-bonded and polyethylene materials, while Loy and Wells (1982) and Gimenez *et al.* (2002) reported 86% transmittance for slitted low tunnels and 65 to 85% for spun-bonded row covers, respectively.

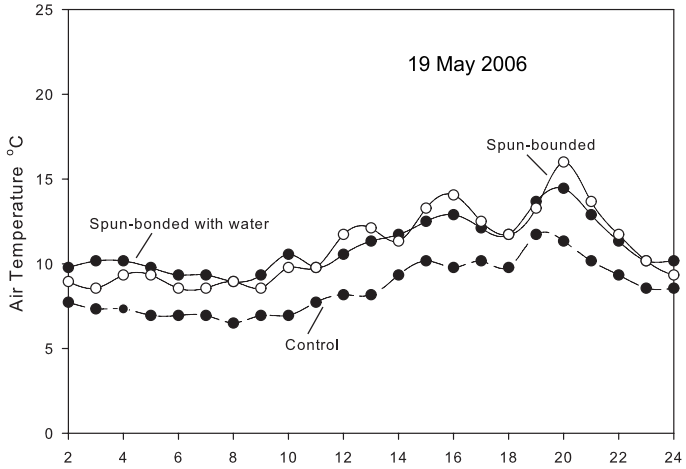

Fig. 3. Hourly air temperatures from 19 May 2006 illustrating the effect of spun-bonded and spun-bonded with water tubes treatments on temperature fluctuations in the plant environment across a spring day.

Total and early yield: Radish and lettuce yields in the spun-bonded row cover and slitted tunnel microclimate treatments were not increased over the control (Table 2). However, the covers did allow earlier planting, which led to harvest occurring twenty-four days earlier than the control in 2006 and fifteen days earlier in 2007. Radish yields in the control plots were higher than the spun-bonded and slitted tunnels without water tubes while the covered treatments with water tubes produced yields similar to the control. The inclusion of water tubes in spun-bonded and slitted covered treatments showed an increase in radish yield in the water tube vs. none contrast (P=0.042). Lettuce biomass was similar in all plots (Table 2). Gimenez, *et al.* (2002) similarly reported spun-bonded floating row covers (17g m^{-2}) not significantly increasing leafy crop yields over those without covers.

In this study, the earlier planting dates of the treatments typically equaled the earlier harvest of cool season crops, meaning that the modified microclimates appeared able to produce crops in a comparable number of days with an earlier spring planting date. In 2006, soil conditions permitted planting eight to ten days earlier than in 2007 and the earliness of yield in the modified microclimate treatments was increased to greater relative degree over the control than in 2007 in these cool season crops. This could illustrate that techniques that modify the microclimates potentially have the greatest relative effect on crop growth when field growing conditions are the most challenging. This topic of when in a season microclimate modification techniques can be used to the greatest advantage likely deserves additional research. In this study, spun-bonded and polyethylene coverings and water tube inclusion techniques were important in controlling the timing of crop harvest in cool season crops by producing comparable yields and allowing similar production schedules in different portions of the spring season.

Pepper total yields were higher in 2007 than in 2006, but treatment effects were similar in the two years and treatment by year interaction was not significant. Pepper total yields (Table 2) were significantly higher than the control for both tunnel treatments that included water tubes, while treatments without the addition of water tubes had yields similar to the control. The spun-bonded and slitted tunnel with water tube treatments produced 138 and 139% of their respective microclimate treatments without water tubes. Tomato yields in the spun-bonded tunnels, spun-bonded

tunnels with water tubes and slitted tunnels with water tubes all showed increased total yields over the control. In addition to higher total yields, the harvest period of bell peppers and tomatoes was extended by four weeks and two weeks, respectively, because of earlier planting dates. Since the cessation of harvest occurred at the same time in all plots, earlier yields in the season contributed to an increased total yield in the microclimate modification treatments.

Early yield (before the first harvest in the control) for tomato and pepper were collected in the days or weeks prior to the appearance of ripe fruit in the control plots in each year. The inclusion of water tubes in microclimate modification treatments significantly increased early yields in the pepper crop (P=0.0065). Spun-bonded and slitted tunnel treatments with water tubes produced 179 and 181% of the yield of their respective treatments without water tubes. In contrast to the pepper early yields, the presence of water tubes in the treatments did not increase tomato early yields. There were also no significant difference between the spun-bonded and slitted polyethylene tunnel treatments for early tomato yields (P=0.077). Early pepper and tomato yields were significantly different with 2007 producing more early pepper yield than 2006. In contrast to peppers, early tomato yields, though not significantly different across microclimate treatments, were higher in 2006 than 2007. These data suggest that early yields are temperature sensitive and could be crop specific; therefore, variable seasonal conditions can be critical in early yield determination.

Increased protection in the tunnel treatments facilitated planting warm seasons crops approximately eighteen days before the control in both years. However, harvest of ripe pepper fruit from the spun-bonded and slitted tunnels began occurring about thirty days prior to the control in both years. In the tomato crop, ripe fruit occurred earlier in the tunnel treatments by generally the same numbers of days as early planting occurred. Therefore, the tunnels were able to allow comparable production schedules when begun earlier in the season under colder temperatures as was seen in the cool season crops, but the tunnels and water tubes also increased the development of plants and maturation of pepper fruit.

Many researchers (Hemphill Jr. and Mansour, 1986; Ibarra *et al.*, 2001; Loy and Wells, 1982; Marr *et al.*, 1991; Soltani *et al.*, 1995; Taber, 1993) have reported early and total yield increased by row cover and low tunnels on muskmelon and watermelon crops. Peterson and Taber (1991) showed the potential for increased early tomato yield under low tunnels if excessively high temperatures were avoided. Gerber *et al.* (1988) and Waterer (1992) reported increased early pepper yields when low tunnels were used in addition to plastic mulch while only Gerber *et al.* (1988) showed increased total yields in the covered treatments. Waterer (1992) also found spun-bonded covers to more consistently increase early yields than slitted polyethylene.

The use of tunnels both with and without water tubes for the purpose of moderating microclimate temperatures and therefore potentially increasing growth and yield of warm season crops has been supported by prior experimentation (Aziz *et al.,* 2001; Jenni *et al.*, 1998). Aziz *et al.* (2001) reported that tunnels with water tubes and proper ventilation could impact relative growth rates and plant dry weight in muskmelon early in the growing season. Rangarajan (1998) and Rangarajan and Ingall (1998) also reported an increase of 30% in bell pepper early yields when clear water tubes were used under low tunnels. Similar results were seen in this study with increased early pepper yield in low tunnel treatments with water tubes. However, over the period of harvest in our experiment, total yield was also increased. Bell peppers are warm season crops that can require even higher temperatures than tomatoes for optimum growth and productivity (Decoteau, 2000). Our research suggests that the effects of covers and water tubes were magnified on this cold sensitive crop. Accelerated growth and production by plants in the low tunnel treatments with water tubes were observed in this study because pepper plants seeded and transplanted at the same time produced significantly higher early yields when water tubes were added to the tunnels.

Economic analysis: Economic analysis showed that all microclimate modification treatments tested have the potential to raise returns and profits for growers. When the materials and labour costs in excess of the control for each microclimate modification treatment are subtracted from the potential returns (yield x market price) from each year (data not shown), a comparison of potential profits emerges (Table 3). Control returns were the lowest in both years while the highest potential returns were observed in covered treatments. The potential economic impact of water filled tubes differed slightly by year and type of low tunnel material. Hemphill Jr. and Mansour (1986) and Waterer (1993) showed that net returns in muskmelon production could often be increased by the use of row covers. Rangarajan (1998) also demonstrated an increased earning potential from using water filled tubes under tunnels for pepper production. The ultimate decision of what techniques to implement depends on the level of investment possible and local market conditions. If cold frames or high tunnels prove too great of an initial investment for growers (Waterer, 2003), row covers incorporating a water buffer could be considered a potentially viable microclimate

Table 2. Average total and early yields per plot (grams 18 m^{-2}). Total yields represent all mature biomass removed from the plot over the harvest season. Early yields represent mature fruit harvested from plots prior to the appearance of mature fruit in the control plots. Treatments with the same letter are not significantly different from each other as separated by LSD at P= <0.05. (N= 6)

Treatment	Lettuce total yield	Radish total yield	Pepper total yield	Pepper early yield	Tomato total yield	Tomato early yield
Control	1300	3255 (a)	2980 (b)	0	5700 (c)	0
Spun-bonded	2083	2342 (b)	4735 (ab)	1427 (b)	14840 (ab)	1192
Spun-bonded with water	2205	2788 (ab)	6526 (a)	2549 (a)	21232 (a)	1497
Slitted	1712	2363 (b)	4778 (ab)	1414 (b)	12576 (bc)	444
Slitted with water	2375	2897 (ab)	6668 (a)	2569 (a)	14763 (ab)	1083
	NS	*	*	*	**	NS
LSD		666	2477	1081	7366	
Control vs. others contrast		*	**	NA	**	
Spun-bonded vs. slitted contrast		NS	NS	NS	NS	
Water tubes vs. no water tubes contrast		*	*	**	NS	

NS, *,** Non significant or significant at P= 0.05,0.01, respectively

Table 3. Estimated net returns for each of the experimental treatments

Parameter/ Year	Control	Spun-bonded	Spun-bonded with water	Slitted	Slitted with water
2006					
Income	35.02	73.99	96.22	76.49	91.30
Added costs**	0	12.32	33.32	10.70	31.70
Net returns*	$35.02	$61.67	$62.90	$65.79	$59.60
2007					
Income	54.58	92.57	128.93	70.19	93.46
Added costs**	0	12.32	33.32	10.70	31.70
Net returns*	$54.58	$80.25	$95.61	$59.49	$61.76

Estimated using actual data on labour, material costs and sales prices. The additional labour and input costs of the treatments were subtracted from the potential returns calculated with yields and actual farmers markets sales price to obtain a measure of how potentially profitable each treatment was in each year of the experiment.

* Net returns = ((early season average yields) x (early season price) + (regular season average yields) x (regular season price)) – (added costs)

** Added costs= (additional labour + spun-bonded and slitted cover, water tubes, wire hoops)

modification alternative.

Based on the observations, methods employed in this study could be considered as useful microclimate modification techniques. Warm season tomato and pepper crops showed increases in early and/or total yield as a function of the use of covers and water tubes while cool season radish and lettuce yields were not higher than the control. However, lettuce and radishes were harvested earlier, which could impact early season marketing. Earlier yields were made possible in all crops because of earlier planting or accelerated growth in modified microclimates. Peppers were the most sensitive crop with respect to early and total yield, and higher yields were seen with the inclusion of water tubes when compared to treatments without water tubes. The addition of water as a temperature buffer could potentially be used with any microclimate modification technique to improve overall and early yields in warm season crops.

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Received: January, 2010 Revised: April, 2010 Accepted: September, 2010

Journal of Applied Horticulture, 12(2): 93-96, July-December, 2010

Changes in inner contents of 'Kyoho' grape berry during the growth and ripening period

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Abstract

The grape berry morphologically consists of epidermis, an outer wall, an inner wall and placenta. The inner contents such as soluble solids, organic acids and moisture distribution of grape berries are dramatically changed during the véraison between the growth and ripening period. However, we know little about the changes in the inner contents of the outer wall, inner wall and placenta. Our purpose of the study is to clarify the tissue specificity of the total soluble solids content, sugar composition, flesh firmness and moisture distribution of 'Kyoho' grape berry during growth and ripening period. The moisture distribution of the grape berries was analyzed with magnetic resonance imaging (MRI). The total soluble solids contents of the outer wall were higher than those of the inner wall during the investigation period. In this study, fructose, glucose and sucrose were detected in the berries. The concentrations of these sugars in the outer wall were higher than those in the inner wall; however, the components of these sugars were not different between the outer wall and inner wall. Even within the same berry, the flesh firmness and the moisture distribution were different from one part of the tissue to another. These results indicate that the growth rate of grape berry varies considerably among the different parts of the berry.

Key words: Flesh firmness, 'Kyoho' grape, magnetic resonance imaging, ripening, sugar contents

Introduction

The seasonal growth pattern of grape berries shows a double sigmoidal pattern that can be divided into two major stages of growth, separated by a lag phase (Coombe, 1992). During stage I, the skin colours of berries are green and the flesh is hard, and an increase in the level of organic acids is observed, while the levels of glucose and fructose are low. Stage II is referred to as the lag phase of berry development. The lag phase is followed by a second major stage of growth called stage III. During stage III, the inner contents such as soluble solids, organic acids and moisture distribution of the grape berries are dramatically changed after the inception of berry ripening, which is called véraison.

There have been reports about the changes in the inner contents of grape berries during the growth and ripening period. Amerine (1956) showed that the titratable acidity of young berries was highest in the skin area and lowest around the seeds. Possner and Kliewer (1985) reported that the highest concentrations of sugars were found in the fruit core and the tissue below the peripheral vascular bundles after véraison. Thus, the location of compounds in the grape berries are different according to the growth stage and specific tissue. Only a few reports, however, have focused on the location of compounds within specific tissues.

Our purpose here is to clarify the tissue specificity of the total soluble solids content, sugar composition and flesh firmness of 'Kyoho' grape berry during the growth and ripening period. Berries were examined non-destructively using MRI to clarify the moisture distributions in the berries during the growth and ripening period.

Materials and methods

Thirteen-year-old vines of 'Kyoho' grape grown at the experimental farm of Shimane University were used in 2005 and 2006. We randomly selected five clusters for monitoring the growth curve of grape berries. The diameters of five berries selected from each cluster were measured from 28 to 77 days after full bloom (DAB) in 2005 and 21 to 77 DAB in 2006, respectively. For total soluble solids content measurement, three clusters were randomly selected, and three berries were sampled from each cluster at 28, 42 (véraison), 49 and 77 DAB in 2005 (Fig. 1). The outer wall and inner wall tissue were excised from each berry with a razor blade (Fig. 2). Juice was squeezed from each tissue and analyzed with a hand refractometer (AMY-1, SHIMADZU Co., Japan). For analysis of flesh firmness of the berries, three clusters were randomly selected, and three berries were sampled from each cluster at 28, 42, 49 and 77 DAB in 2005. The flesh firmness was measured as previously described by Mori (2000) with some modification. A vertical slice (4 mm thickness) was excised with a razor blade from the equatorial part of each berry. Four points of the vertical slice were tested with a rheometer (COMPAC-100, Sun Scientific Co., Ltd., Japan) (Fig. 3). The tip angle of the probe was 30 deg., and the inserted depth was 3.9 mm. For MRI, two clusters were randomly selected, and two berries were sampled at 28, 42, 63 and 77 DAB in 2005. Magnetic resonance images of each berry were collected in a MRI system (MAGNETOM Symphony 1.5T, Siemens AG., Germany).

In 2006, three clusters were randomly selected, and five berries were sampled from each cluster at 49 (7 days after véraison),

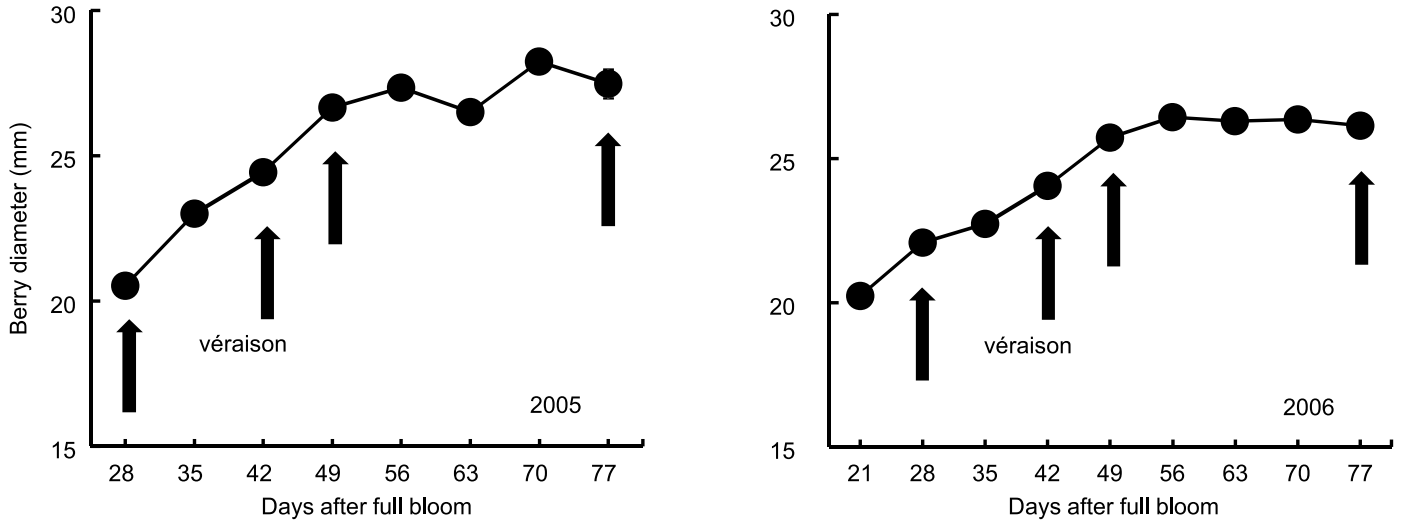

Fig. 1. Changes in diameter of 'Kyoho' grape berries during the growth and ripening period. The arrows indicate the sampling date.

63 and 77 DAB for analyzing of sugar components of the juice. Samples of the outer wall and inner wall tissue were excised with a razor blade from each berry. Juice was squeezed from each tissue and filtrated through a membrane filter (0.45 μm, CSO45AN, Toyo Roshi Kaisya, Ltd., Japan). The juice was diluted with distilled water and subjected to HPLC analysis. Twenty microlitres of the sample was injected to the HPLC system equipped with a pump (L-2130, Hitachi High-Technologies Co., Japan), a column (Wakosil, $5NH_2$, φ4.0mm×200mm, Wako Pure Chemical Industries, Ltd., Japan), a column oven (L-2350, Hitachi High-Technologies Co., Japan) and a refractive index detector (RID-10A, SHIMADZU Co., Japan). The column temperature was 40˚C, and 80% acetonitrile was used as an eluent at a flow rate of 1 mL min^{-1}. Each sugar was identified by the retention time and then quantified from a standard curve using an authentic sugar.

Results

Total soluble solids content: During the investigation period, the total soluble solids content of the outer and inner wall samples gradually increased (Table 1). The total soluble solids contents of the outer wall were significantly higher than those of the inner wall.

Flesh firmness: During the investigation period, the flesh firmness gradually decreased (Table 2). At 28 and 42 DAB, the flesh firmness was highest around the seeds (Fig. 3, area III and IV) and decreased from the berry centre to the skin. However, there were no differences in flesh firmness between the areas at 49 and 77 DAB.

Sugar composition: In this study, fructose and glucose were detected in the juice from the outer and inner wall samples at 49, 63 and 77 DAB (Table 3). In contrast, sucrose was only detected at 77 DAB. The concentrations of these sugars gradually increased during the investigation period and were significantly higher in the outer wall than in the inner wall.

Magnetic resonance images: In the case of transverse sections, the areas of the outer wall, inner wall and placenta were clearly seen as regions of uniform gray signals at 28 DAB (Fig. 4A). After 42 DAB, the signals of the outer wall were lighter than those of the inner wall (Fig. 4B, C and D). This tendency was more obvious

Table 1. Changes in the total soluble solids contents of outer wall and inner wall of 'Kyoho' grape berries during the growth and ripening period

Fruit part	Days after full bloom			
	28	42	49	77
Outer wall	6.0 ± 0.4	11.0 ± 0.5	18.8 ± 0.3	25.6 ± 0.3
Inner wall	5.2 ± 0.2	9.9 ± 0.6	18.1 ± 0.3	25.2 ± 0.3
Significance	*	*	**	*

*,**Significant at P= 0.05 or 0.01, respectively by t-test.

Table 2. Changes in the flesh firmnesses (g) of 'Kyoho' grape berries during the growth and ripening period

Fruit part[z]	Days after full bloom			
	28	42	49	77
I	47.6c[y]	14.1c	14.7[NSx]	12.4[NS]
II	55.4bc	24.1bc	12.1[NS]	9.3[NS]
III	73.2a	32.2ab	11.2[NS]	8.8[NS]
IV	69.4ab	39.8a	13.1[NS]	13.4[NS]

[z]Refer to Fig. 3.
[y]Different letters within a column indicate significance at 5% level by the Tukey's test. [x]Non significant.

Table 3. Changes in the concentration (mg mL^{-1} juice) of fructose, glucose and sucrose in the outer wall and inner wall of 'Kyoho' grape berries during the growth and ripening period

Sugars	Fruit part	Days after full bloom		
		49	63	77
Fructose	Outer wall	67.64	123.00	141.74
	Inner wall	47.68	111.83	133.12
Significance		*z	*	*
Glucose	Outer wall	68.76	100.83	127.84
	Inner wall	51.79	93.35	123.11
Significance		*	*	*
Sucrose	Outer wall	ND[y]	ND	7.88
	Inner wall	ND	ND	6.05
Significance				*

[z]Significant at 5% level by t-test. [Y]ND indicates not detected.

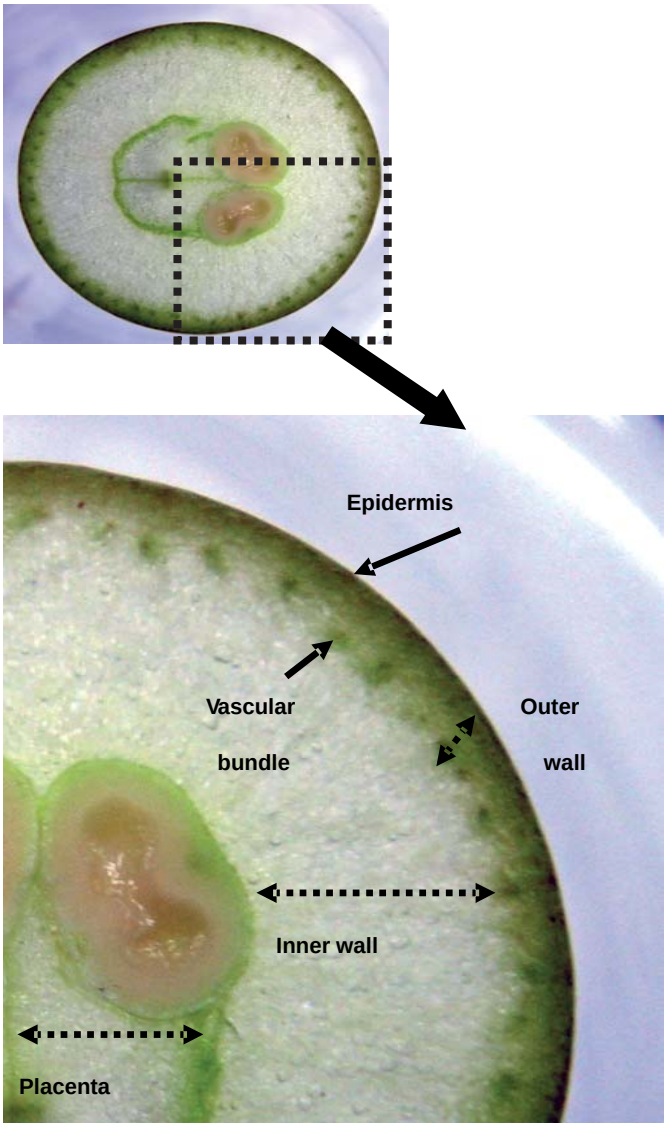

Fig. 2. Transverse section through a 'Kyoho' grape berry.

in the images at 77 DAB (Fig. 4D). At 77 DAB, black signals were clearly observed in the inner wall of a berry.

Discussion

The developing grape berry represents a strong sink for photosynthesis products (Hale and Weaver, 1962). In the grape vine leaf, sucrose is produced as a result of photosynthesis and transported via the phloem to the berry (Swanson and Elshishiny, 1958). The photosynthesis products are mainly transported via the peripheral and central vascular systems in the berries

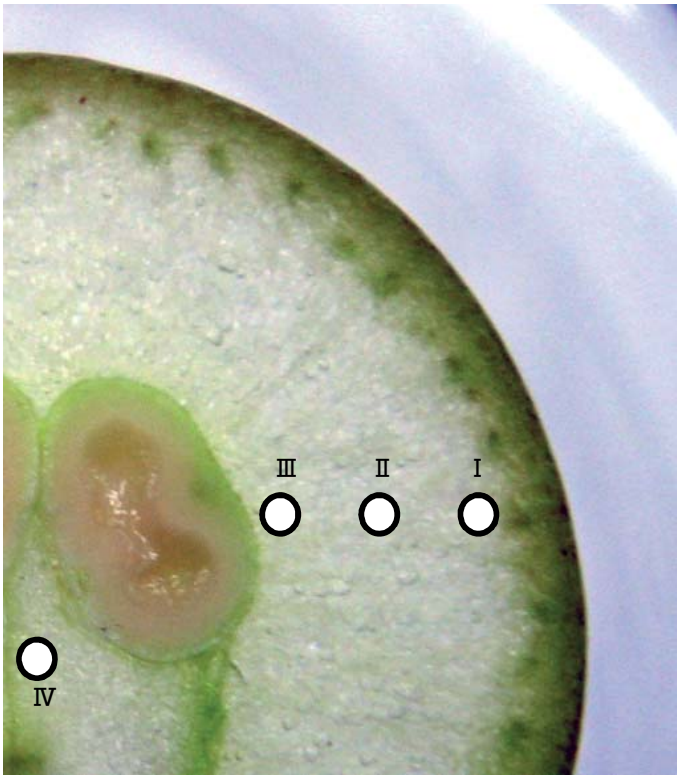

Fig. 3. The four points of a 'Kyoho' grape berry tested with a rheometer.

(Kriedemann, 1969). Possner and Kliewer (1985) discussed how these vascular systems caused the localization of sugars in grape berries. In this study, the total soluble solids, fructose, glucose and sucrose contents of the outer wall were significantly higher than those of the inner wall during the investigation period (Table 1 and 3). The peripheral vascular system of a grape berry is presented between the outer and inner walls, and the inner wall tissue is thicker than the outer wall tissue (Fig. 2). The localization of these photosynthesis products in the outer and inner wall tissue is probably correlated to the thickness of these tissues, depending on the distance from the peripheral vascular system.

The grape berry rapidly softens at the beginning of stage III, and the flesh firmness gradually decreases toward the harvesting time. To clarify this softening mechanism, researchers have mainly investigated the changes in the cell wall materials of flesh tissue (Nunan *et al.*, 2001; Yakushiji *et al.*, 2001). However, the changes in the flesh firmness of each part of a grape berry are still unknown. The results of the present study clearly show that there was a gradual decrease in flesh firmness from the core of a berry towards the skin during the growth period, whereas there

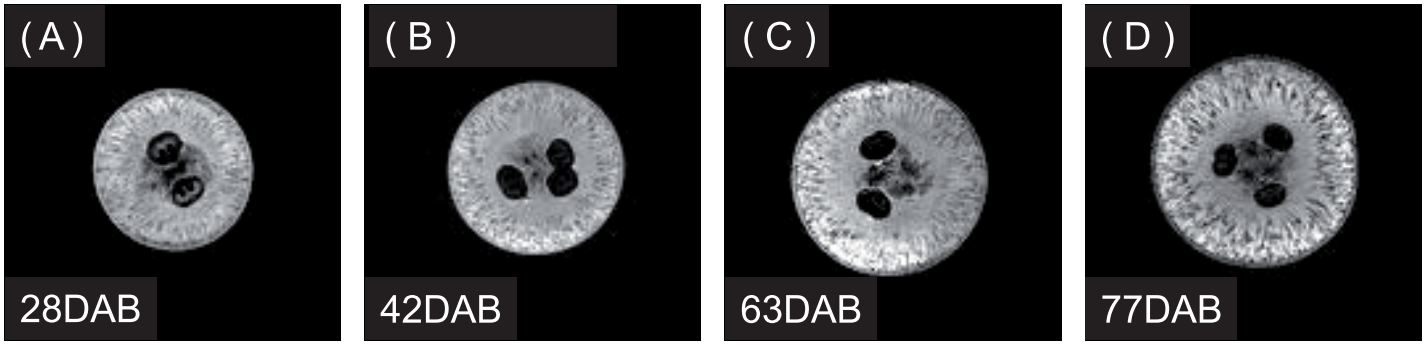

Fig. 4. Proton MR images of transverse sections through a 'Kyoho' grape berry.

were no differences in flesh firmness among the different parts of a berry after véraison (Table 2). These findings indicate that the rate of decreases in flesh firmness varies from one part of the grape to another. It is possible that the differences in flesh firmness of grape berries before véraison can be attributed to the tissue structure.

MRI is a nondestructive technique usually used in medical sciences, which examines the distribution and mobility of protons (mainly from water and fat) in living tissue (Ishida *et al.*, 1989). The concentration and state of water and fat play very important roles in plant tissues when active metabolism occurs. MRI techniques have been used to investigate the changes of physiological activities along with maturation of some fruit, such as apples (Barreiro *et al.*, 1999), citrus (Galed *et al.*, 2004), grapes (Pope *et al.*, 1993) and persimmons (Clark and MacFall, 2003). However, little is known about the changes in the water distribution of grape berries during the growth and ripening period. In this study, MR images clearly detected the differences in the distribution of water during the investigation period (Fig. 4). Even in samples from the same areas of a berry harvested at 77 DAB, the signal intensities of MR images were different from each other (Fig. 4D). These differences indicate the uneven distribution of water in a grape berry.

Ishida *et al.* (1989) analyzed tomato fruits with MRI and reported that the water was differently distributed among the tissues in the fruits. They concluded that the distribution of free water involves the places where active metabolism occurs and that low mobility resulted in slow metabolism. Since the water content of grape berry tissue do not differ among the different parts of the tissue, these results may indicate the differences in the degree of active metabolism in a berry. Further investigation is required to clarify the mechanisms of uneven water distribution in a grape berry.

In conclusion, we found that the sugar concentrations in the outer wall of a grape berry were higher than those of the inner wall. We also noted that the flesh firmness and the moisture distribution were different in each part of the grape tissue. These findings suggest that there is uneven distribution of the inner contents in a grape berry and that the growth rate of berries is quite different from one part of the tissue to another.

Acknowledgments

The authors thank Mr. Shinichi Hino and Mr. Michio Kato of Matsue Seikyo Hospital for technical assistance with MRI experiments. This work was supported, in part, by a Grant-in-Aid (No. 17380023) from the Japan Society for the Promotion of Science.

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Received: February, 2010 Revised: July, 2010 Accepted: September, 2010

Journal of Applied Horticulture, 12(2): 97-101, July-December, 2010

Optimization of guava edible coating using response surface methodology

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Abstract

Application of edible coating represents a method that can extend the shelf life of picked guava by minimizing the loss of weight mainly due to natural migration process of moisture and gases. Response surface methodology (RSM) was employed to search for best composition of edible coating which comprised of three variables namely palm stearin, palm olein and beeswax. Based on central composite rotatable designs of RSM and weight loss as response, 15 coating compositions were established involving 8 factorial points, 6 axial points and 1 centre point. From the RSM-generated model, optimum coating composition for minimizing guava weight loss was identified as palm stearin 4.5% (w/v), palm olein 1% (v/v) and beeswax 1% (w/v). Under this optimum composition, the predicted weight loss of coated guava was 7.18%, whereas, the experimental weight loss of coated guava was 7.51% after tenth days of storage period. The RSM-predicted and experimental weight loss were not significantly different from each other. The weight loss of uncoated guava was 3 times higher (25%) after 8 days of storage as compared to coated guava. Thus, the use of optimum composition of edible coating provides acceptable alternative for post harvest control of weight loss of guava during storage.

Key words: Response surface methodology (RSM), guava, stearin, olein, beeswax, weight loss

Introduction

Guava (*Psidium guajava*) is a tropical, climacteric fruit that ripens rapidly and is highly perishable. The shelf-life ranges from 3 to 10 days at room temperature (Campbell, 1994; Vazquez-Ochoa and Colinas-Leon, 1990). If the guavas are kept without any treatment, they may be spoiled mainly due to loss of water from fruit surface, faster respiration rate, attack of microorganisms and development of physiological disorders. The loss of weight in guava is due to migration of moisture, volatile compounds and gases such as oxygen, carbon dioxide and ethylene.

To minimize the post harvest losses of guavas, edible coating can be applied. There are many advantages of fruit coating, among which are retaining freshness, firmness and colour. Lipid-based coatings are generally more effective barriers to moisture while polysaccharide-based coatings are generally good gas barriers (Hagenmaier and Shaw, 1990; Kester and Fennema, 1988). The coating may be applied by dipping or drenching or for experimental purposes by brushing (Smith *et al.*, 1987).

Palm olein and palm stearin are produced by fractionation of palm oil after crystallization at controlled temperature. Palm stearin is a by-product of palm oil, inexpensive and has limited usage. One of the current technological concerns of the edible oil industry is how to expand the multiple usages of palm oil and its by-products. The use of palm stearin and palm olein as part of coating composition can be justified by their constituents of palmitic, oleic and stearic acids. According to Morillon *et al.* (2002), palmitic acid and stearic acid have very low water vapour permeability (0.65 and 0.22 x 10^{-12} g $m^{-1}s^{-1}$ Pa^{-1}), respectively. The criterion of water vapour permeability is important because it may reflect the ability of coating to act as a moisture barrier. Beeswax, a product from bee hive, has a lowest water vapour permeability of 0.006 x 10^{-12} g m^{-1} s^{-1} Pa^{-1}. With this value, beeswax has many applications in food, cosmetic and pharmaceutical industries.

RSM originally described by Box and Wilson (1951) enables evaluation of the effects of many factors and their interactions on response variables. The advantages of using RSM are reported to be the reduction in the number of experimental runs needed to evaluate multiple variables, and the ability of the statistical tool to identify interactions (Chen *et al.*, 2004; Lee *et al.*, 2000). Therefore, it is less laborious and less time-consuming compared to one-variable at a time. RSM has been widely applied for optimizing conditions and processes in various food studies (Junqueira *et al.*, 2007; Liyana-Pathirana and Shahidi, 2005).

In this study, all lipid-based coating ingredients *i.e.*, palm stearin, palm olein and beeswax were emulsified with water to form coating emulsion and subsequently applied on fruit surface by dipping technique. The central composite rotatable design from RSM was used as a statistical method to optimize edible coating composition comprised of stearin, olein and beeswax in minimizing the weight loss of guava.

Materials and methods

Guavas: Samples of fresh guavas were obtained from a commercial farm, Sui Yuan Fruit Trading, in Bidor, Perak, Malaysia. Guavas were carefully selected (maturity index 2) to obtain uniformity based on size, shape, colour and absence of injuries. The fruits were packed in small boxes and transported to the laboratory in Shah Alam, Selangor, Malaysia. Pre-treatment of guavas were made according to the method applied by Soares *et al.* (2007). Selected guavas were unwrapped, washed and sanitized in potassium sorbate 0.5% for 2-3 minutes and left to dry at ambient conditions (25-27°C, RH 80-90%).

Coating ingredients: Refined, bleached and deodorised palm kernel olein and palm stearin with a slip melting point of 49.6°C and iodine value 40.3 were obtained from Cargill Palm Products Pte. Ltd., Port Klang, Malaysia. Beeswax (Sigma-Aldrich, GmbH, Sternheim, Germany) was purchased form a local supplier. All other chemical and ingredients used were either of analytical or food grade.

Preparation and application of coating emulsion: Coating emulsion was prepared conceptually similar to the method applied by several researchers (Rojas-Graü *et al.*, 2008; Tapia *et al.*, 2007). Beeswax was heated up to 90°C in distilled water while stirring on hot plate (Fisher Scientific, USA) until the solution became clear. Palm stearin, palm olein and 0.5% (v/v) emulsifier (Tween 40, Sigma-Aldrich, GmbH, Sternheim, Germany) were added immediately followed by high speed mixing using an Ultra Turax T10 (IKA®, Germany). Guavas were labelled and marked before being dipped in coating emulsion at 60°C for 15 seconds and left to dry at ambient conditions. Weight measurement was performed on daily basis until day 10 of storage period.

Weight loss: Weight loss was determined by the difference between the initial and final weights of each replicate. Percentage of weight loss was calculated as follows:

$$\text{Weight loss (\%)} = \frac{\text{(Initial weight)} - \text{(Final weight)}}{\text{(Initial weight)}} \times 100$$

Experimental design and statistical analysis: Edible coating composition affecting weight loss of guava was optimized using Design-Expert version 6.0.6 RSM software (Stat-Ease Inc., Minneapolis, USA). Each variable was examined at five different levels (relatively low, low, basal, high, relatively high) coded (--, -, 0, +, ++) as shown in Table 1. The design required 48 runs derived from 15 combinations of the independent variables performed in random order, including replicates of the centre region and factorial points. The obtained responses were subjected to an analysis of variance, R-square and were evaluated for lack of fit (LOF). Accordingly, an equation in terms of coded of second-order polynomial could be calculated of the type:

$$Z = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad \text{Equation 1}$$

where, Z was the dependent variable (weight loss); A, B and C were the independent variables for palm stearin, palm olein and beeswax respectively; β_0 was the regression coefficient at centre point; β_1, β_2 and β_3 were linear coefficients; β_{12}, β_{13} and β_{23} were second-order interaction coefficients; and β_{11}, β_{22} and β_{33} were quadratic coefficients.

Optimum composition was obtained using the optimization module of RSM software. The experimental and predicted values were compared in order to determine the validity of the developed model. Verification of model was performed similar to the method applied by Tan *et al.* (2009).

Results and discussion

Model fitting and analysis of response: The response was percentage of weight loss in which a good composition should minimize the weight loss of guava within 10 days of storage period. Details about experimental runs, coating composition and response of weight loss are given in Table 1.

Table 1. Central composite rotatable design for three independent variables: palm stearin (A), palm olein (B) and beeswax (C)

Standard	Run	A: palm stearin (g)	B: palmolein (g)	C: Beeswax (g)	Z: Weight loss (%)
1	22	2 (-)	2 (-)	2 (-)	9.87
2	12	2 (-)	2 (-)	2 (-)	10.05
3	18	2 (-)	2 (-)	2 (-)	11.87
4	15	5 (+)	2 (-)	2 (-)	9.06
5	26	5 (+)	2 (-)	2 (-)	8.50
6	3	5 (+)	2 (-)	2 (-)	9.10
7	19	2 (-)	5 (+)	2 (-)	10.32
8	38	2 (-)	5 (+)	2 (-)	11.33
9	31	2 (-)	5 (+)	2 (-)	11.51
10	5	5 (+)	5 (+)	2 (-)	10.77
11	20	5 (+)	5 (+)	2 (-)	10.02
12	7	5 (+)	5 (+)	2 (-)	11.90
13	17	2 (-)	2 (-)	5 (+)	14.73
14	11	2 (-)	2 (-)	5 (+)	12.15
15	6	2 (-)	2 (-)	5 (+)	13.41
16	25	5 (+)	2 (-)	5 (+)	10.77
17	23	5 (+)	2 (-)	5 (+)	11.23
18	13	5 (+)	2 (-)	5 (+)	11.68
19	32	2 (-)	5 (+)	5 (+)	8.65
20	9	2 (-)	5 (+)	5 (+)	9.16
21	43	2 (-)	5 (+)	5 (+)	8.06
22	40	5 (+)	5 (+)	5 (+)	7.65
23	35	5 (+)	5 (+)	5 (+)	9.54
24	21	5 (+)	5 (+)	5 (+)	7.98
25	36	0.98 (--)	3.5 (0)	3.5 (0)	12.98
26	44	0.98 (--)	3.5 (0)	3.5 (0)	12.53
27	42	0.98 (--)	3.5 (0)	3.5 (0)	12.15
28	39	6.02 (++)	3.5 (0)	3.5 (0)	10.90
29	14	6.02 (++)	3.5 (0)	3.5 (0)	10.76
30	45	6.02 (++)	3.5 (0)	3.5 (0)	9.87
31	46	3.5 (0)	0.98 (--)	3.5 (0)	12.07
32	8	3.5 (0)	0.98 (--)	3.5 (0)	11.65
33	48	3.5 (0)	0.98 (--)	3.5 (0)	10.43
34	33	3.5 (0)	6.02 (++)	3.5 (0)	7.76
35	1	3.5 (0)	6.02 (++)	3.5 (0)	7.53
36	2	3.5 (0)	6.02 (++)	3.5 (0)	6.81
37	27	3.5 (0)	3.5 (0)	0.98 (--)	8.23
38	34	3.5 (0)	3.5 (0)	0.98 (--)	7.76
39	29	3.5 (0)	3.5 (0)	0.98 (--)	9.68
40	30	3.5 (0)	3.5 (0)	6.02 (++)	7.62
41	41	3.5 (0)	3.5 (0)	6.02 (++)	8.47
42	24	3.5 (0)	3.5 (0)	6.02 (++)	9.56
43	37	3.5 (0)	3.5 (0)	3.5 (0)	8.82
44	4	3.5 (0)	3.5 (0)	3.5 (0)	9.09
45	16	3.5 (0)	3.5 (0)	3.5 (0)	9.22
46	10	3.5 (0)	3.5 (0)	3.5 (0)	8.62
47	47	3.5 (0)	3.5 (0)	3.5 (0)	8.66
48	28	3.5 (0)	3.5 (0)	3.5 (0)	8.47

Symbols (--, -, 0, +, ++) indicates relatively low, low, basal, high and relatively high.

Analysis of variance (Table 2) of the model revealed that all selected terms had significant effects on the response. This was indicated by the small value of probability for F value (less than 0.05). However, the term C (or beeswax) did not have significant effect on response. According to Myers and Montgomery (1995), non-significant term can be dropped from a model or fixed at one level. Thus, we decided to fix beeswax at low level (1% w/v) in the coating composition. Furthermore, the interaction between B and C (palm olein and beeswax) showed a significant result on response. Meanwhile, LOF test measured variation of the data around the fitted model. If the model did not fit the data well, LOF

test would be significant. In this case, the selected model showed non-significant results. A model should be rejected if the results showed significance in the LOF test (Myers and Montgomery, 1995).

Table 2. Analysis of variance of the model terms

Source	Sum of square	df	Mean square	F value	Prob > F	Status
Model	125.97	7	18.00	29.27	<0.0001	Significant
A	13.16	1	13.16	21.40	<0.0001	Significant
B	31.27	1	31.27	50.87	<0.0001	Significant
C	0.011	1	0.011	0.018	0.8935	NS
A2	38.54	1	38.54	62.68	<0.0001	Significant
B2	3.16	1	3.16	5.13	0.0290	Significant
AB	4.66	1	4.66	7.57	0.0089	Significant
BC	38.33	1	38.33	62.34	<0.0001	Significant
Residual	24.59	40	0.61			
Lack of fit	5.69	7	0.81	1.42	0.2308	NS
Pure error	18.90	33	0.57			
Cor total	150.57	47				

NS: Non significant

The selected model comprised of several important properties as listed in Table 3. According to Joglekar and May (1987), the coefficient of variance (CV) should be less than the standardized value of 10%. The CV value of the selected model was 7.86% which was below that of the standardized value. The predicted residual sum of square (PRESS) value measures how the model fits each point in the design. The lower the value of PRESS, the better the model fits the point. In our case, when the term AC was added the PRESS value increased to 38.37, reflecting the term AC should be dropped from the model. For the model fitted, the coefficient of determination (R^2), which is a measure of degree of fit (Haber and Runyon, 1977), was 0.8367. This implied that 83.7% of the variations could be explained by the fitted model. Joglekar and May (1987) suggested that for a good fit of a model, R^2 should be at least 0.80. Adjusted R-Square was used to measure the amount of variation around the mean which was adjusted for the number of terms in the model. The

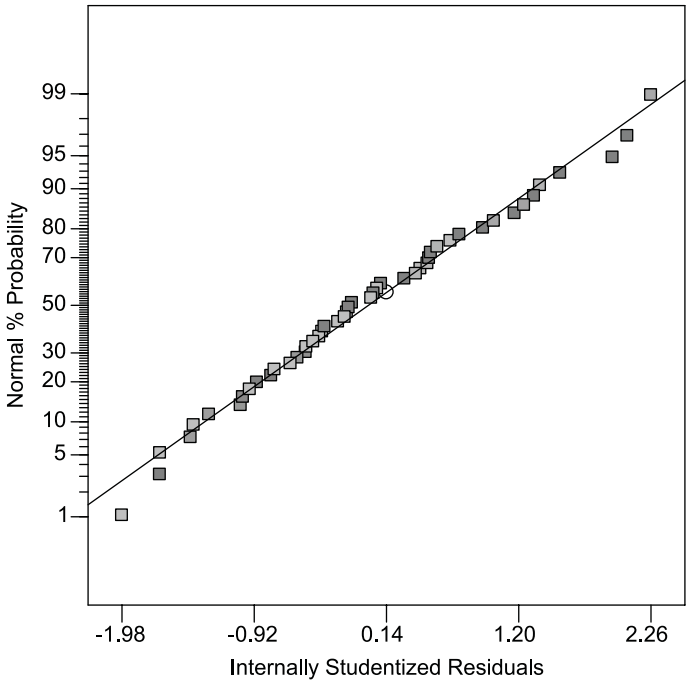

Fig. 1. All points scattered on straight line in normal plot of residuals for the selected model.

Table 3. Collection of summary of statistics for the selected model

Parameters	Value
Standard deviation	0.78
Mean	9.98
C.V	7.86
PRESS	36.27
R-Squared	0.8367
Adjusted R-Squared	0.8081
Predicted R-Squared	0.7591
Adequate Precision	16.895

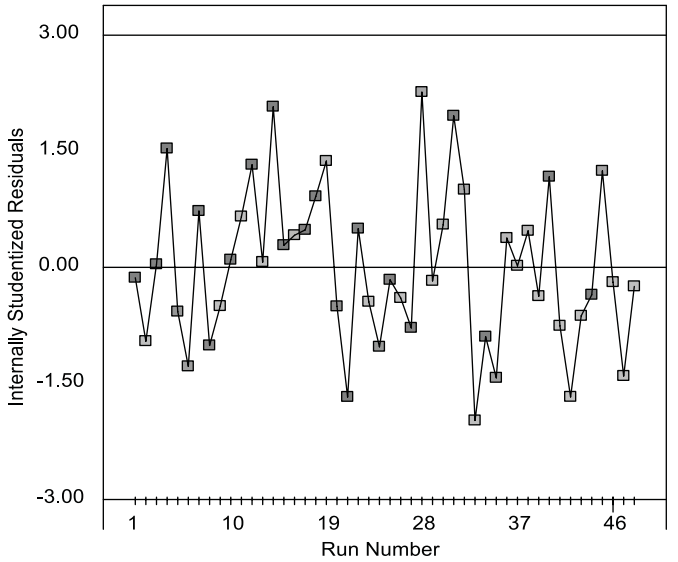

Fig. 2. All points scattered randomly in this plot of residual against experimental run number.

adjusted R-Square decreased as the number of terms in the model increased if those additional terms do not add value to the model. For instance, when the term AC was added, the adjusted R-Square decreased from 0.8081 to 0.8041, which indicated that the term AC did not contribute significantly to the response. The adequate precision value of the selected model was 16.9. Adequate precision measures the signal to noise ratio and a ratio greater than 4 indicates an adequate signal (Stat-Ease Inc., Minneapolis, USA). Thus the model can be used to navigate the design space.

The selected model can also be interpreted into a coded equation as shown in Equation 2. The equation is used to correlate a relationship between the three variables and percentage of weight loss.

$$Z = 8.81 - 0.57A - 0.87B + 0.017C + 1.07A^2 + 0.31B^2 + 0.31B^2 + 0.44AB - 1.26BC \quad \text{Equation 2}$$

Diagnostic and optimum coating composition: Normal probability plot indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line. The results showed that the points were scattered along the straight line (Fig. 1) and no bad shape like an "S-shape" was observed. Plot residual against run was used to check lurking variables that may have influenced the response during the experiment. The plot should show a random scatter as in Fig. 2. Similar pattern and shape were observed in the plot outlier against run number (the plot is not shown but is similar to Fig. 2) which indicated no outliers and all points were randomly scattered within plus or minus 3.5 (standard value set by the software).

Fig. 3 expressed the relationship in the form of a three dimensional plot between palm stearin and palm olein when level of beeswax

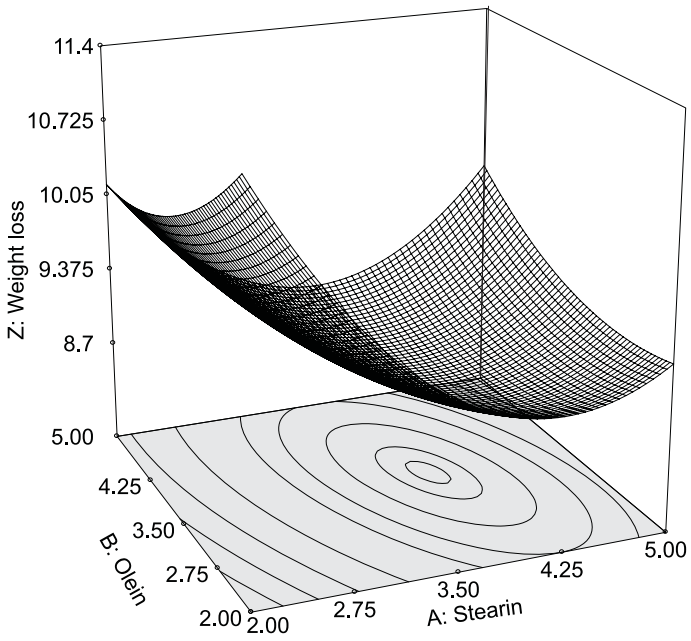

Fig. 3. Three-dimensional plot showing relationship between fruit weight, palm stearin and palm olein when level of beeswax set at 2.61% (w/v).

was set at 2.61% (w/v). It can be observed that the weight loss decreased to a lowest area of about 8% as palm stearin increased from 2 to 4% (w/v). However, the weight loss increased to about 8 to 10% as palm stearin increased from 4.3 to 5% (w/v).

The optimization module was evaluated with the aid of software (Stat-Ease Inc., Minneapolis, USA) by a combination of variables levels that simultaneously satisfy the requirements placed on the response and variables. Based on economic reason, we decided to set the minimum goal for beeswax at 1% (w/v). The goals for both palm stearin and palm olein were set in the range of study. Beside that, the lower limits of variables were extended backward from 2-5 to 1-5% as permitted by the software. Results showed that the optimum composition for minimizing guava weight loss was identified as palm stearin 4.5% (w/v), palm olein 1% (v/v) and beeswax 1% (w/v). Using this optimized coating composition, the predicted response was 7.18% weight loss of guava after 10 days of storage period.

Model verification: Model verification was performed by additional four sets of independent trials using the mentioned composition and *t*-test was carried out to determine the validation of the experimental results as compared to the predicted value from RSM. The predicted value suggested by the module and experimental results gave identical values which indicate that the results of validation parameters were reliable and satisfactory. In this study, RSM successfully optimized the coating composition used to minimize weight loss of coated guava compared to uncoated guava. Thus, the use of optimum composition provides acceptable alternative for post harvest control of weight loss of guava during storage.

Optimized coating composition was used to compare the weight loss with uncoated guava (Fig. 4). Both coated and uncoated guava showed a linear increase in weight loss percent against days of storage. However, weight loss for uncoated guava was faster (eight-fold) compared to that of coated guava (five-fold) within 10 storage days. On day 8, the weight loss was about 25% for uncoated guava. Mello Prado *et al.* (2005) reported the weight

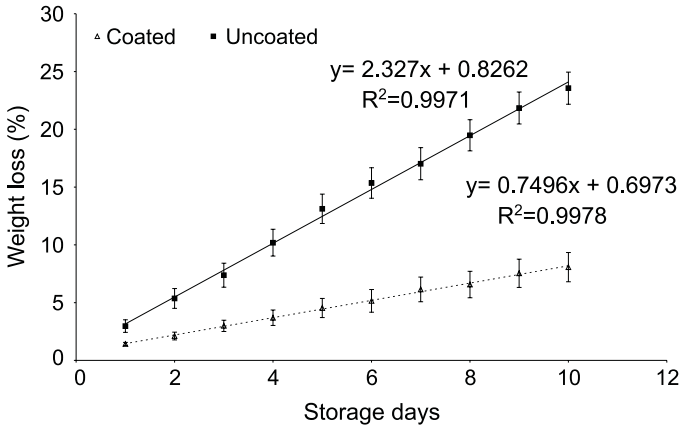

Fig. 4. Fruit weight loss for uncoated and coated guava using optimized composition of stearin 4.5% (w/v), olein 1% (v/v) and beeswax, 1% (w/v).

loss of guava (cv. Paluma) reached 18 and 22% on day 7 and 8, respectively, while Singh and Chauhan (1982) reported an 18% weight loss with guava cv. L-49 after 8 days of storage at room temperature. However, Adsule and Tondon (1983) observed that the weight loss of guava (cv. Allahabad) reached 30%. These differences occurred due to the variation in climatic conditions and the cultivars used by the authors.

The study revealed that weight loss of uncoated guava was 3 times higher after 8 days of storage as compared to coated guava. Thus, the use of optimum composition of edible coating provides acceptable alternative for post harvest control of weight loss of guava during storage.

Acknowledgements

Thanks are due to University of Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology (UniKL-MICET), Melaka and Science Fund of Malaysian Government (Project number: 03-01 – 01-SF0033) for financial support. Also, thanks to Cargill Palm Products Pte. Ltd., Port Klang, Malaysia, for providing RBD palm stearin and RBD palm olein.

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Received: December, 2009 Revised: April, 2010 Accepted: August, 2010

Journal of Applied Horticulture, 12(2): 102-106, July-December, 2010

Effect of pine bark, pine straw and red oak amendments on pH of potting medium

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Abstract

Our objective was to determine temporal effects on medium pH caused by decomposition of three organic amendments incorporated with topsoil. Pine (*Pinus taeda* L.) bark, pine (*Pinus taeda* L.) straw, and red oak (*Quercus falcata* Michx. var. *falcata*) were ground to uniform particle size, incorporated with a silt loam topsoil at two rates (1:29 and 1:10 amendment:soil, w:w basis, referred to as 1X and 3X, respectively), placed into greenhouse pots, and sampled during 12 months to determine medium pH in comparison to an unamended topsoil (control). Compared to the control, pine straw, pine bark, and red oak 3X increased soil medium pH. All media except pine straw increased pH during the study. At any given sampling date, pine straw 3X had lower pH than the control, while red oak either did not differ from, or had higher pH than the control. By the end of the sampling period, pine bark and pine straw media had lower pH than the control. While statistically significant, change in medium pH caused by any of these substances would be trivial for most horticultural crops, and easily corrected by use of other liming or acidifying amendments.

Key words: Growth media, *Pinus taeda*, *Quercus falcata*, soil amendments

Introduction

Components of a potting mixture are often selected based on their cost, availability, and physical and chemical effectiveness in mixture (Bilderback, 1982). About one-third to one-half of the total volume of the growing medium is occupied by solids and organic matter, the rest is pore space (Bilderback, 1982). The organic matter constituent(s) has numerous beneficial effects on soil quality, including storage and supply of plant nutrients, stabilization of soil aggregates, aids water infiltration and retention, and improves soil porosity (Brady, 1974). Of the array of possible organic choices, pine bark, pine straw, and hardwood chips are commonly-used, surface-applied mulches. Pine bark and sawdust also are commonly used sources of organic matter as soil-incorporated amendments. Milled pine bark and sawdust are acidic in the raw state (Starbuck, 1994; Thomas and Schumann, 1993), but their effect on soil pH as amendments has not been sufficiently investigated. It is commonly assumed that pine straw mulch acidifies soil (Meyer, 1997), perhaps because of observations at forest scale. Loblolly pine (*Pinus taeda* L.) plantations exhibit a decrease in topsoil pH after decades of tree growth (Adams *et al.*, 1999; Markewitz *et al.*, 1998), with long-term decreases as great as 1 pH unit (Richter *et al.*, 1994).

Short-term effects of organic amendments on soil pH are contradictory. Anecdotal information suggests that freshly-fallen oak (*Quercus* L. sp.) leaves and pine needles are relatively more acidic than some other common forest tree species. (http://asecular.com/forests/phleaves.htm, verified 20 January 2010), and that garden applications of sawdust, composted leaves, and wood chips will lower the soil pH (http://www.thegardenhelper.com/acidsoil.html, verified 20 January 2010). A 9-cm thick layer of surface-applied slash pine (*P. elliottii* Engelm.) straw caused a decrease in topsoil pH from 5.0 to 4.4 after 1 year (Duryea *et al.*, 1999). Longleaf pine (*P. palustris* Mill.) straw mulch caused a reduction of 0.56 pH units when surface-applied in two consecutive years on one soil (a total of 16.5 t ha^{-1}), but a single application of 11 t ha^{-1} loblolly pine straw on another soil caused no change in pH after one year (Makus *et al.*, 1994).

Mulches typically have large particle size and are surface applied, so it is difficult to compare their decomposition effects on soil properties to those of incorporated amendments. The objective of this experiment was to determine temporal effects on medium pH caused by decomposition of three organic amendments incorporated with a silt loam topsoil. Our hypothesis was that amendments would not significantly affect medium pH compared to the control.

Materials and methods

The experiment was conducted in potting containers near Booneville, AR (35° N, 94° W, 150 m above sea level). This was a randomized complete block design with seven amendment-rate (media) treatments, eight sampling dates, three replications within repetitions, and two repetitions. The two repetitions of the experiment were conducted concurrently. There were three fresh (not composted) amendments used in the experiment: pine bark [a mixture of bark and sapwood of loblolly pine], loblolly pine straw, and sawdust of southern red oak. Amendments were air dried (20 to 30 °C) and ground to 2 mm particle size in a Wiley mill. The topsoil was a Leadvale silt loam (fine-silty, siliceous, thermic Typic Fragiudult) freshly obtained from the surface 8 cm of a field site cultivated with wheat (*Triticum aestivum* L.). Air dried topsoil, ground in a mortar to 1.4 mm, and amendments were blended in a clean cement mixer.

There were three topsoil-amendment rates: control (no amendment), 1X, and 3X rates. The control rate (topsoil) was equivalent for each amendment, so only one control treatment was retained. The treatments consisted of control, pine bark at 1X and 3X rates, pine straw at 1X and 3X rates, and red oak at 1X and 3X rates (336 containers total). The 1X rate was equivalent to 39 Mg ha^{-1} of surface-applied amendment incorporated (to hasten decomposition) into topsoil to a 15-cm depth (Starbuck, undated; Taylor and Foster, 2003). Amendments were mixed with topsoil using a cement mixer. The 1X and 3X rates were equivalent to 1:29 and 1:10 ratios (w:w) of amendment:topsoil, respectively. Containers were 9.5 cm square (350 mL capacity) and were filled about 7-cm deep with designated medium mixture. Excess water was allowed to exit through drainage holes in the bottom of each container.

Containers were placed on metal greenhouse benches in the field under a loblolly pine tree canopy. The shaded environment provided a cooler, more humid environment during summer than could be achieved in the greenhouse. A removable, porous weed barrier fabric (PAK Masterscape 475, Hummert Int., Earth City, Missouri) was draped loosely across the containers to retain moisture, allow rainfall penetration and air exchange, exclude fallen pine needles from the tree canopy from contaminating the treatments, and exclude light to prevent weed growth. Soil was kept moist by removing the fabric and applying tap water to each container at least twice weekly.

Air temperature and rainfall, measured 1.4 m above soil surface, were continuously recorded at 0.5 h intervals from March 2005 through March 2006 at an unofficial weather station located in a meadow adjacent to the experimental site. Long-term (1971 to 2000) air temperature and rainfall data were obtained from an official weather station (NOAA, 2002) located 2.6 km from the experimental site for comparison.

Sampling dates were t_0 (23 March 2005), 30 and 60 d after initiation (t_1 and t_2, respectively), and every 60 d thereafter (120 d (t_3), 180 d (t_4), 240 d (t_5), 300 d (t_6), and 360 d (t_7)] for 12 months. There were 42 containers collected at each sampling date. Pots were removed from the bench at designated intervals, media was air dried (20 to 30°C), and ground in a mortar to 1.4 mm. To minimize laboratory error, samples were completely randomized at the termination of the experiment and medium pH was measured on duplicate samples in a single-blind manner in comparison to a reference pH standard. Media samples and distilled water (25°C) were mixed in a 1:2 soil:water ratio (w:v), and manually stirred with a glass rod for 20 min. before measuring the slurry pH.

Individual amendments were analyzed at t_0 for C, N, mineral (Ca and Mg) composition, and pH. Carbon and N were analyzed by combustion (Elementar Vario Macro, Hanau, Germany). Concentrations of Ca and Mg were determined by HNO_3 digestion and detection by inductively coupled plasma spectrometry (University of Arkansas Agriculture Diagnostics Laboratory, Fayetteville, Arkansas). The pH was measured on a 1:4 amendment:water (w:v) slurry. Tap water was analyzed at t_7 for Ca, Mg (University of Arkansas Water Quality Laboratory, Fayetteville), and pH.

Analyses of variance of spatially replicated media pH data were conducted using a mixed linear model, Proc Mixed (Littell *et al.*, 1996; SAS Inst., 2002). There were no missing data. Fixed effects were amendment-rate (6 df), sampling date (7 df), and the amendment-rate x sampling date interaction (42 df). Sampling date within repetition and amendment-rate was analyzed as a repeated measure with a variance component covariance structure (Littell *et al.*, 1996). Random effects were repetition, replication within repetition and amendment-rate, and sample date within repetition and amendment-rate. Main amendment-rate effects were compared by single df contrasts. Means of the amendment-rate x sampling date interaction were separated using the Tukey HSD test at $P \leq 0.05$ using (SAS Inst., 2002). Amendment-rate x sampling date interaction responses were also analyzed by regression (SAS Inst., 2002).

Results

For any given month, mean monthly air temperature (but not rainfall) during the 2005-2006 study period was similar to the long term mean (Fig. 1). Rainfall was less than the long-term mean for any given month during the study period, except in September 2005 and March 2006.

Initial C:N ratios were 340:1, 20:1, and 250:1 for pine bark, pine straw, and red oak, respectively, while unamended topsoil had a C:N ratio of 20:1. Further, pine bark, pine straw, and red oak had pH 4.38, 4.14, and 4.82, respectively. Pine bark, pine straw, and red oak had Ca concentrations of 3.2, 6.2, and 1.1 g kg^{-1}, respectively, and Mg concentrations of 0.5, 0.7, and 0.2 g kg^{-1}, respectively. Tap water had pH 7.21, and had Ca and Mg concentrations of 17.2 and 8.09 mg L^{-1}.

Analysis of variance indicated that amendment-rate, sampling date, and the amendment-rate x sampling date interaction significantly affected medium pH ($P \leq 0.001$). At t_0, before irrigation, the control (pH 6.59) had higher pH than most other media ($P \leq 0.05$), while the pine bark 3X (pH 5.49) and pine straw 3X (pH 5.18) amendments had the lowest media pH. Further, pine bark 3X and pine straw 3X had lower medium pH at t_0 than their respective 1X treatments. This suggested that medium pH at t_0 was generally related to pH of the pure amendments before mixing (except for red oak 1X), because all the amendments were considerably more acidic (pH $\leq$ 4.82) than the control soil (pH 6.59).

Some amendments caused a rapid decrease in medium pH from t_0 to t_1: pine straw (both rates), pine bark (both rates), and red oak 3X decreased medium pH at t_1 compared to the control (Table 1). As at t_0, this appeared to have been a function of pH of the unmixed amendments. Further, the red oak 1X and 3X rates did not differ from each other in their effects on pH ($P > 0.05$) at t_0, but the 3X rate had lower pH (6.05) than the control.

At any given sampling date, pine straw 3X had lower pH than the control, while red oak either did not differ from, or had higher pH than the control. Mean pH of pine straw and pine bark 1X media did not differ ($P \geq 0.05$) from the control until t_7 (300 d incubation). At t_7, pH of the pine straw 1X medium was 0.48 units lower than that of the control, and the pine bark 1X medium was 0.40 units lower than the control. The pine straw 3X medium had a lower pH than the control at each sampling date, and also had lower pH than the pine straw 1X medium at each sampling

date except t_3 and t_4. From t_1 through t_7, the control (pH ≥ 6.94) and/or red oak (pH ≥ 6.74) media consistently had higher mean pH values than that of the pine straw 3X medium (pH ≤ 6.57). At t_7, red oak media did not differ from the control, although at t_4 and t_5 pH was higher in the red oak 1X medium (pH ≥ 7.26) than in the control (pH ≥ 6.83). By the end of the sampling period, pine bark and pine straw media had lower pH than the control. Thus, pine straw at either rate, and to lesser extent pine bark, had an acidifying effect, while red oak generally had little effect on medium pH compared to the control.

During the study, pH increased in all media, except pine straw ($P \geq 0.06$) in (Fig. 2). Regression analysis of the amendment-rate x sampling date interaction showed that the control and pine bark 1X media had linear responses with time, pine straw 1X tended ($P = 0.06$) to have a linear response, and the other treatments had quadratic responses with time. Shape of the quadratic responses suggested that the pH increase may have been transient, perhaps decreasing to the t_0 level at some future time.

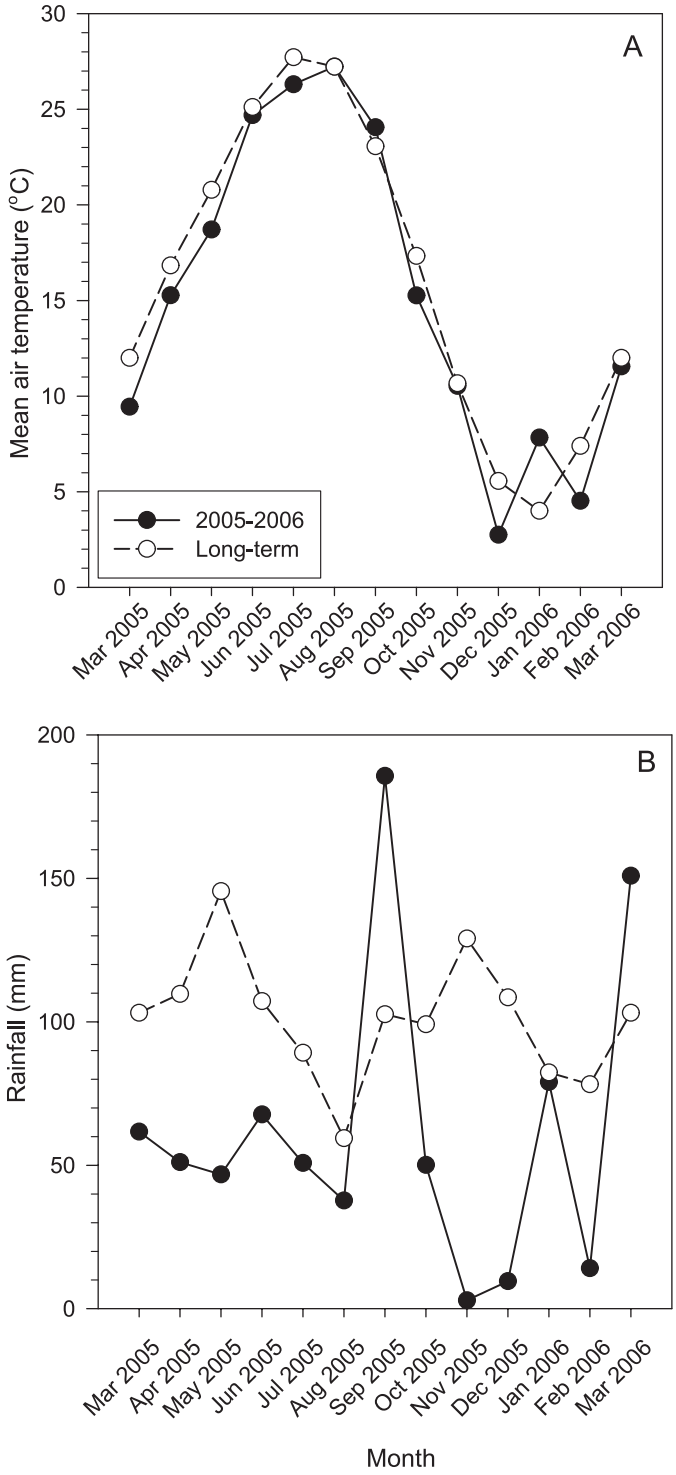

Fig. 1. Mean monthly air temperature (A) and total rainfall (B) at an unofficial weather station located in a meadow adjacent to the experimental site in 2005 and 2006 (solid circles). The long-term means (open circles) for the period 1971 to 2000 were from an official station located 2.5 km from the experimental site near Booneville, Arkansas (NOAA, 2002).

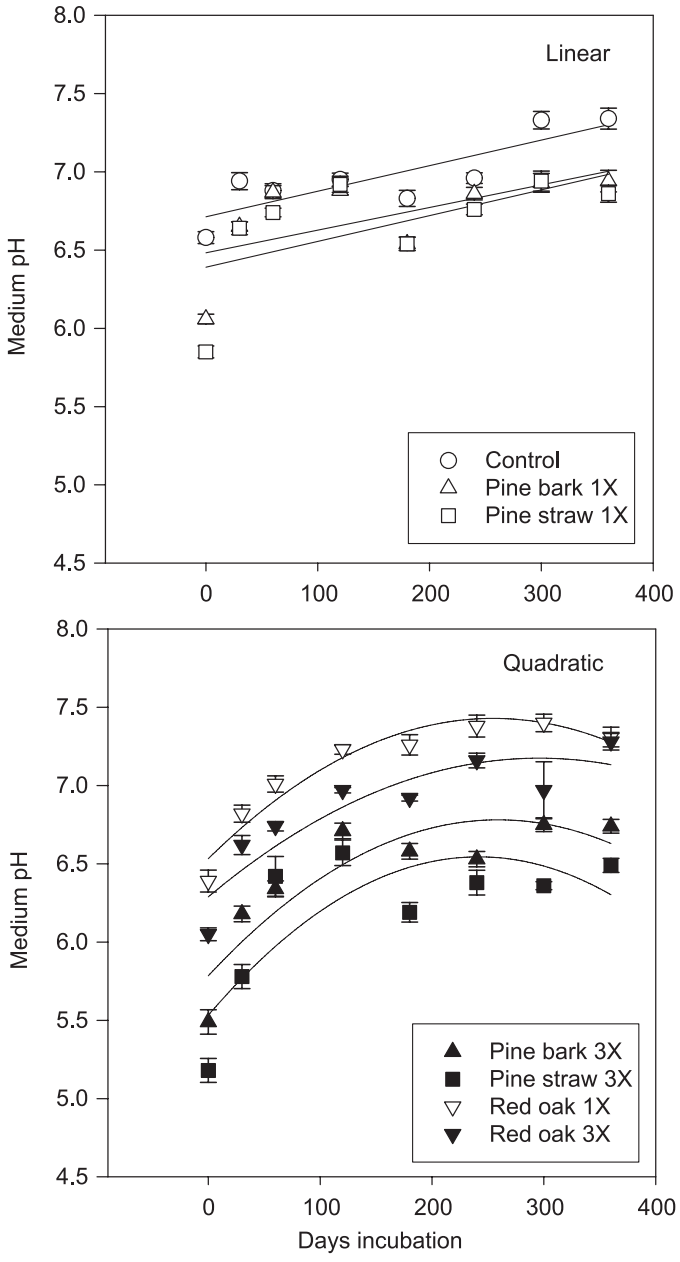

Fig. 2. Effect of amendment-rate on medium pH during 360 d sampling period. Linear regression responses were Control, $Y = 6.67 + 0.0871X$, $R^2 = 0.73$; Pine bark 1X, $Y = 6.42 + 0.0856X$, $R^2 = 0.49$; Pine straw 1X, $Y = 6.31 + 0.0982X$, $R^2 = 0.46$. Quadratic regression responses were Red oak 1X, $Y = 6.43 + 0.354X - 0.0327X^2$, $R^2 = 0.99$; Red oak 3X, $Y = 6.18 + 0.315X - 0.0253X^2$, $R^2 = 0.89$; Pine bark 3X, $Y = 5.64 + 0.415X - 0.0388X^2$, $R^2 = 0.88$; and Pine straw 3X, $Y = 5.34 + 0.486X - 0.0498X^2$, $R^2 = 0.79$. Equations were significant at $P \leq 0.05$, except for Pine straw 1X ($P = 0.06$). Vertical bars at data points are SE ($n = 12$).

Table 1. Temporal changes in pH of a silt loam soil amended with two rates (1X and 3X[a]) of three organic constituents

Sampling period	Sampling date (d)	Control	Pine bark		Pine straw		Red oak	
			1X	3X	1X	3X	1X	3X
t_0	0	6.59a[b]	6.06bc	5.49d	5.85c	5.18d	6.39ab	6.05bc
t_1	30	6.94a	6.65a	6.18b	6.64a	5.78c	6.82a	6.62a
t_2	60	6.88a	6.87a	6.34c	6.74ab	6.42bc	7.01a	6.74ab
t_3	120	6.95ab	6.88abc	6.71bc	6.92abc	6.57c	7.23a	6.97ab
t_4	180	6.83bc	6.54cd	6.58bcd	6.54cd	6.19d	7.26a	6.92ab
t_5	240	6.96bc	6.86bc	6.53cd	6.76c	6.38d	7.38a	7.16ab
t_6	300	7.33a	6.93b	6.75b	6.94b	6.36c	7.40a	6.97b
t_7	360	7.34a	6.94b	6.74bc	6.86b	6.49c	7.31a	7.28ab

[a] 1X and 3X rates correspond to 1:29 and 1:10 ratios (w:w) of amendment: topsoil, respectively.

[b] Means within rows followed by a common letter do not differ at $P \leq 0.05$ using the Tukey HSD test (SAS Inst., 2002).

Discussion

We studied the effect on medium pH when pine bark, pine straw, and red oak amendments were ground and incorporated with mineral soil. Some forest by-products have considerable economic value to the horticultural industry as landscape mulches, but their decomposition effects on soil properties are not always clear. Loblolly pine straw decomposes very slowly on the soil surface, and has about 55% of its initial mass after 26 months (Piatek and Allen, 2001). Surface-applied oak sawdust mulch did not decrease soil pH one year after application (Starbuck, 1994). Pine bark and red oak amendments might have slow decomposition rates given their high C:N ratios (http://www.compostinfo.com/tutorial/ElementOfComposting.htm, verified 20 January 2010; Starbuck, 1994). Pine straw had the same C:N ratio as the topsoil, so it should decompose more rapidly than the other amendments. High C:N ratio and slow decomposition can limit soil N availability (Starbuck, 1994).

Contrary to expectation, there was a transient increase in pH regardless of amendment which might have been affected by the basic pH (7.21) of the tap water. However, even rainfall varies in pH. Rainfall (pH 5.6 to 8.3) at El Reno, Oklahoma (400 km west of the experiment location) tends to be more basic than 'pure' rain (pH 5.6, Smith *et al.*, 1984). Purified water might have been preferred to tap water to eliminate this potentially confounding factor from the study.

Soil amended with various leaf litters increased linearly in pH during an 8 week incubation, in direct relationship to tissue $CaCO_3$ concentrations (Noble and Randall, 1999). It seemed unlikely, however, that the early, transient increase in media pH was solely due to media Ca and Mg concentrations. Concentrations of these cations were relatively low ($\leq$ 6.2 g kg^{-1}) in the amendments, especially in red oak. The Ca and Mg concentrations of pine straw were roughly comparable to those reported by Wells *et al.* (1975).

Any of these organic materials could be used to amend potting soil. We rejected our hypothesis that amendments would not significantly affect medium pH. Pine straw and pine bark amendments decreased media pH by as much as 0.48 units. Red oak generally had no effect on pH compared to the control. While statistically significant, changes in pH caused by pine straw or pine bark amendments were trivial for most horticultural crops, and could easily be corrected by liming. However, the pH decrease could be exacerbated if other commonly-used amendments such as NH_4NO_3, $Al_2(SO_4)_3$, and elemental S were included in the media formulation.

In conclusion, media blends consisting of 1 part amendment to 10 or 29 parts soil caused little change in medium pH during 12 month incubation. Results suggested that pH effects on horticultural crops should be trivial and easily corrected by use of other liming or acidifying amendments. It also seemed unlikely that any of these mulches would substantially alter soil pH when surface applied at typical landscaping rates.

Acknowledgments

Loblolly pine straw was donated by the Booneville Human Development centre (Booneville, Arkansas). Pine bark was donated by Deltic Lumber Mill, Ola, Arkansas. The authors appreciate the technical assistance of K. Chapman and J. Whiley (USDA-ARS, Booneville, Arkansas), and helpful review comments by Drs. Richard Parish (LSU AgCenter, Hammond, Louisiana) and Chris Starbuck (University of Missouri, Columbia). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Received: January, 2010; Revised: April, 2010; Accepted: July, 2010

Journal of Applied Horticulture, 12(2): 107-112, July-December, 2010

Vapour heat quarantine treatment for Taiwan native mango variety fruits infested with fruit fly

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Abstract

The objective of the research was to evaluate the efficacy of Vapour heat treatments (VHT) to disinfest the Taiwan native mango variety fruits (Tuu Shien) from the oriental fruit fly (*Dacus dorsalis* Hendel) and the effect of the treatments on the quality of mango fruits. The three stage treatment of forced air at 30°C for 30 minutes, 30 to 48°C for 60 minutes, and then 48°C forced hot air with saturated humidity over the mango fruit surface until the fruit centre temperature reached 46.5°C and fruit was held for 40 minutes. Survival tests showed that both second and third generation instars were more susceptible to the VHT than eggs and there were no surviving oriental fruit fly after 46.5°C for 40 min. The quality of local mango fruits treated with VHT and stored at ambient temperature (28 ± 3°C) for 6 days was not significantly different from the control.

Key words: Vapour heat, oriental fruit fly, quarantine pests, 'Tuu Shien' mango

Introduction

Mango (*Magifera indica*) is one of the most economically important tropical fruits in the world in term of both worldwide production and cultivated area (Castrillo *et al.*, 1992). In Taiwan, mango is a major fruit crop with high potential for export. The oriental fruit fly, *Dacus dorsalis,* is a direct pest of the mango. Quarantine heat treatment to disinfest oriental fruit fly is required by Taiwan's developing mango industry. Taiwan's mango exports to Japan increased from 481 metric tons in 2005 to 787 metric tons in 2007 and export value was increased from 78.4 million NT$ in 2005 to 131.3 million NT$ in 2007 (COA, 2008).

There are some methods to heat fruit to temperatures beyond the target quarantine pest thermal tolerance limit. Hot air has been used for both fungal and insect control and to study the response of commodities to high temperature. Heat is transferred from the air to the fruit by condensation of water Vapour (heat of condensation) on the relatively cooler fruit surface. The technique consists of a period of warming which can be faster or slower depending on a commodity's sensitivity to high temperatures. Fruit may be gradually heated over time to a desired temperature that may be either the treatment end temperature, or a holding temperature maintained for a specific time required to kill all target pests. For mango disinfestation, treatments can only utilize heat, because of the strong sensitivity of this fruit to cold temperatures. The heat treatments, in general, consist of using an immersion in hot water by a system of batches or an uninterrupted bath. Heat can also be obtained by use of forced hot air or hot vapour, because temperatures higher than 45°C kills fly eggs and larvae (Marie-Noëlle *et al.*, 2007). These treatments may then be followed by a fast cooling fruit system which can be carried out by ventilation with cold air or hydro-cooling.

Beside, a commercial 800 watt-microwave oven was used to heat on export-graded 'Chokanan' mango (average mass of 0.32 kg/ fruit). It was found that mango heated at 50% microwave power for 40 seconds yielded an internal temperature of 45°C at 23 mm underneath the skin (Varith *et al.*, 2006). However, even though a drawback of microwaves is non-uniform heating, knowing nature of the microwaves and where the waves concentrate in the mango will help overcome uneven heating problem and assist with the process design (Varith *et al.*, 2006). On the other hand, the treatment of mangoes with microwaves depends much on fruit shape and size. The mango fruit is more or less a compressed, fleshy drupe. It varies considerably in size, shape, colour, flavour, taste, presence of fiber and several other characteristics. The shape of the fruit varies from rounded to ovate-oblong or longish, with the length varying from 2.5 to 30 cm according to variety. So the efficiency of microwave treatment will depend on how the fruit is placed.

The latest technology to control fruit fly infestations in mangoes is the extended hot water treatment (EHWT), a cheaper alternative to the expensive vapour heat treatment (VHT). In EHWT, the fruits are dipped 10 cm below the surface of the heated water at a temperature of 48°C. Pulp temperature at 46°C is held up to 15 minutes and a calibrated thermometer sensor - is used to monitor the pulp and water temperature. Four sensors are attached from the computer to the fruits to record at what time the 48°C-fruit fly killing temperature was reached (Golez, 2009).

The common method used to control fruit fly in mangoes is VHT. However, VHT was developed specifically for insect control. VHT of fruit is air saturated with water vapour at temperatures of 43 to 46.5°C at which insect eggs are killed. This procedure was first used to kill Mediterranean (*Ceratitis capitata* Wiedemann) and Mexican (*Anastrepha ludens* Loew) fruit fly (Hawkins, 1932; Baker, 1952) in a chamber without forced air. In modern facilities, the vapour heat includes forced air which circulates through the pallets and heats the commodity more quickly than vapour heat without forced air.

'Tuu Shien', a Taiwan native mango variety, is a green skin, yellow flesh and small-sized fruit. It has the longest history of cultivation and the largest cultivation area in Taiwan. It was introduced into Taiwan 400 years ago. When 'Tuu Shien' mango fruit are exported to Japan, it is necessary to subject fruit to quarantine heat treatment. This study is to find an optimum procedure of vapour heat treatment for 'Tuu Shien' mango fruit.

Materials and methods

Plant material: The test Taiwan native mangoes (Tuu Shien) were purchased from a local market during the mango season in 2009, and then brought to the Horticultural Research Laboratory, Department of Horticulture, National Chung Hsing University, the night before vapour heat treatment. Mangoes were infested with oriental fruit fly and used in vapour heat treatment tests. Fruits that were of an uniform size, weighing an average of 120–130 g, at horticultural maturity stage (based on external visual colour and size) with an absence of visible wounds were selected.

Vapour heat treatment: The VHT is a quarantine method of heating fruit with air saturated with water vapour at temperatures in the range of 40-50°C, according to the procedure developed by Animal and Plant Health Inspection Service (APHIS, 1985). The three stage treatment of forced air at 30°C for 30 minutes, 30 to 48°C for 60 min, and then 48°C forced hot air with saturated humidity is circulated over the mango fruit surfaced until the fruit centre temperature reaches 43 or 46.5°C, depending on treatment aims. In the heat tolerance test of oriental fruit fly in mango fruit inoculated for 0, 2, 4, and 6 days were subjected to the VHT until fruit centre temperature was 43°C for 15 and 30 minutes. In the small-scale disinfestation test inoculated mangoes were subjected to VHT until fruit centre temperature was at 46.5°C for 10, 20, 30, 40, and 50 minutes. In the large-scale disinfestations test, inoculated mangoes were subjected to VHT until fruit centre temperature was 46.5°C for 40 minutes.

Eggs of oriental fruit fly: Fruit fly species used in fruit infestations were the oriental fruit fly. The oriental fruit flies were collected annually from infested guava orchards in Chang-hua county (central Taiwan) guava orchards. Wild oriental fruit flies were mixed in with them in order to sustain the population's natural characteristics. The fruit flies used in this study had been kept in the Taichung branch office, APHIS, for 9 generations and fruit fly eggs used to infest mango were collected. The collected eggs were placed in a beaker and provided with water and oxygen for preservation before use in testing. Some of the collected eggs were also preserved for 20 to 30 hours by evenly spreading them over a wet, black cotton cloth and keeping them at room temperature 25 ± 2°C. Eggs were kept on a wet black cotton cloth and were placed in Petri dish in groups of 50 eggs. Then groups of 50, 100, 150, 200 eggs were determined by a chronometer and injected into mango fruits.

Oriental fruit fly eggs infestation method: A knife was used to slice a 1.5 cm triangular cut on the face of each mango. A piece of pulp of the same volume was removed, but the peel of the mango was retained to recover the hole. Oriental fruit fly eggs were injected below the fruit surface in each triangular hole. After the process of egg injections was finished, a piece of sticking-plaster was used to cover the hole. The infested mango fruits were enclosed in loosely tied white nets to prevent the escape of maggots. They were placed in plastic baskets (35 x 30 x 7 cm) then the baskets were put into plastic trays. Lastly, the top of trays were tightly covered by black cloths. The trays were placed in room temperature for incubation until the larvae developed to the desired stage.

The test for density of oriental fruit fly in mango: Oriental fruit fly eggs (50, 100, 150, and 200 per hole) were separately inoculated into the triangular hole cut into mango fruits. Seven days after the inoculation, 3 mango fruits from every batch were selected to determine the number of live larvae. Larvae were counted to determine the number hatched during the 7 day incubation period.

The test for development of oriental fruit fly in mango: Fifty oriental fruit flies were separately inoculated into the triangular hole on mango fruits. On each of the 8 days after the inoculation, the 5 mango fruits were cut to collect larvae and the developing stages were identified. First and second-instar larvae were distinguished according to the presence of a front spiracle. Second and third-instar larvae were identified by the size, shape, and length of their oral hooks. For each day, the results were counted and recorded for larvae rate and growth stage.

Heat tolerance test of oriental fruit fly in mango fruit: To test the heat tolerance of oriental fruit fly, vapour heat treatment was used. A batch of 100 oriental fruit fly eggs was inoculated into mango. All of the fruits were placed in baskets according to the time duration of its VHT treatment. The infested mangoes were divided into 5 groups and incubated at room temperature for 0, 2, 4 or 6 days. Then they were subjected to VHT until fruit centre temperature held at 43°C for either 15 or 30 minutes. Control was no heat treatment. After VHT finished, the baskets were taken out, wrapped in white netting, cooled, and kept at room temperature. Finally, mortality rates for each treatment were calculated, vapour resistance for each immature stage was compared.

The test for small-scale disinfestations: The mango fruits were treated with 48°C vapour heat until the fruit centre temperature reached to 46.5°C and then held for 10, 20, 30, 40 and 50 min, respectively. In the small-scale test, each fruit was artificially inoculated with 100 eggs, the then placed into groups of thirty fruits for each treatment. There were 6 treatments for time including control. After vapour heat, the fruits were kept at room temperature for 7 days. The larvae were then collected by rinsing the fruits with water over screen filters. The larvae were counted after development to the third-instar. The number of survivor and mortality rate were counted and recorded. The results of the disinfestation test were determined.

The test for large-scale disinfestations: This test for large-scale disinfestation follows the method as the same small-scale disinfestations tests. However, a mango fruit was inoculated with 200 oriental fruit fly eggs in two holes. They were divided into a control and one large test group. The large test group was subjected to vapour heat treatment until fruit centre temperature was 46.5°C for 40 minutes, removed to room temperature for 7 days and then counted. The number of survivor and mortality rate also were counted and recorded.

Quality analysis: Ninety sample mangoes were taken from the

original seven hundred ninety three mangoes for quality analysis. They were placed into 2 jars, ten mangoes each and, 0, 3, and 6 days after VHT, were evaluated for colour, firmness, and total soluble solid.

Colour and decay: Decay development was assessed by viewing the mango fruits' skin. A colour index was recorded according to the following rating scale (Shorter and Joyce, 1998): 1 = 100% green; 2 = 75% green; 3 = 50% green and 50% yellow; 4 = 75% yellow; 5 = 100% yellow.

Firmness (N): Firmness measurements were taken by Rheo meter (Sun Rheo Meter, Sun Scientific, Japanese) as the force required for a 3 mm stainless steel probe to penetrate the cut surface of mango fruit held perpendicular to the probe. Firmness was reported as force in newtons (N).

Total soluble solid (TSS): Immediately after firmness and colour measurements were made, samples were stored at room temperature (25°C) till TSS analysis. Juice samples were prepared by throughly mixing mango slices (two slices for every mango fruit). Total soluble solid was assessed with a Digital Hand-held "Pocket" Refractometer PAL-1 and expressed as a percentage.

Statistical analysis: The experiments were conducted in a completely randomized design. The mean values were analyzed by SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance was performed; means were compared by the least significant difference and Tukey tested at P=0.05 and 0.01.

Results and discussion

Effect of artificial inoculation density of eggs on the hatch rate of oriental fruit fly: Different densities of oriental fruit fly eggs were used to test for hatch rate (from 50, 100, 150, and 200 eggs, respectively). After artificial inoculation and incubation at 25°C for 7 days, the highest hatch rate was about 73% in 100 eggs/hole treatment (Table 1). There was no significant difference of hatch rate between treatments (eggs/hole). However, when inoculation density was increased the hatch rate decreased. The results indicated that 100 eggs/hole is optimum density. The rates of oriental fruit fly larvae development were not significantly different between treatments. After 7 days, all larvae had developed to the 3rd stage (Table 1).

The development of oriental fruit fly in 'Tuu Shien' mango fruit: Forty mangoes were divided into 8 groups of 5 fruits and each mango was injected with 50 oriental fruit fly eggs. Table 2 shows that according to the rearing time for each group of 'Tuu Shien' mango fruit, larvae rate varied from day to day. The results were used as a basis for distinguishing eggs stage and larvae stage, including the first (1st), second (2rd), and third (3nd) instar larvae. The eggs quickly hatched and those that did not immediately hatch to larvae were reduced from 61.6 to 3.6% on the first day and the second day after inoculation, respectively. On the 1st day after incubation, 38.4% of the eggs had hatched and developed to first instar. The hatching rate increased to 96.4% on the second day. The instar developed to second stage after 3 days of incubation and reached third stage on the fourth day. By the seventh day, 26.7% of the eggs became pupa. However, the rate was only 1.9% on the sixth day. In all groups, over half of the inoculated eggs developed into larvae (Table 2).

Table 1. Effect of density of eggs in artificial inoculation on the hatch rate of oriental fruit fly, in which the inoculated mango fruits were stored at 25±2 °C for 7 days

Treatments[y] (Eggs/hole)	Larvae (Numer of hole)	Hatch rate (%)	Development stage
50	36.0	72.0	2.93
100	73.0	73.0	3.00
150	92.3	61.6	2.83
200	93.3	50.7	3.00
Mean		64.3	2.94
F-value[z]		NS	NS
CV (%)		14.2	5.58

[z]F-value for main effect or interaction significant at P>0.05. [y]Fly eggs were placed inside the hollowed area (1 cm^2) of the fruit.

According to growth conditions in which the oriental fruit flies were reared, the duration of the egg stage was determined more than 24 hours after egg inoculation. On the other hand, both 1st and 2rd-instar larvae were found on the third day after inoculation. Similarly, the larvae rate for 2rd and 3nd-instar were increased, and it was the highest after 5 days of inoculation of oriental fruit fly eggs. It must be noted that the number of 3rd-instar larvae was higher than other developmental stages after 6 days of egg inoculation. The number of pupa was highest after 8 days of egg inoculation (Table 2). This demonstrated that, after injection of oriental fruit fly eggs into mango fruit, approximately 6 days was needed in order for eggs to change to pupae (Table 2). The development stage for the 3rd-instar larvae of oriental fruit fly on carambola fruits was determined after 5 days of inoculation (Chang *et al.*, 2009).

In addition, there was no difference in larva length after 5, 7, and 8 days, while in the other treatments larva length increased with rearing time (data not shown). The highest degree of larva

Table 2. The development stages of oriental fruit fly in 'Tuu Shien' mango fruit, the infested fruits were inoculated with 50 eggs per fruit and stored at 25±2 °C for 8 days

Rearing time	Egg (rate)	1st instar (rate)	2rd instar (rate)	3rd instar (rate)	Pupa (rate)	Larva length (mm)
1 day	30.8 (61.6)	19.2 (38.4)				0.24c[x]
2 days	0.8 (3.6)	21.2 (96.4)				0.54b
3 days			24.6 (100)			0.70b
4 days			2.0 (7.4)	25 (92.6)		0.90a
5 days			3.0 (9.0)	30 (91.0)		0.98a
6 days				31 (98.1)	0.6 (1.9)	1.08a
7 days				27.4 (73.3)	10 (26.7)	1.00a
8 days				10.4 (44.1)	13.2 (55.9)	0.96a
Mean						0.80
F-value						***
CV (%)						11.68

[x]Values in columns followed by the same letter are not significantly different according to the Tukey test of transformed data. F-value for main effect or interaction significant at P<0.001

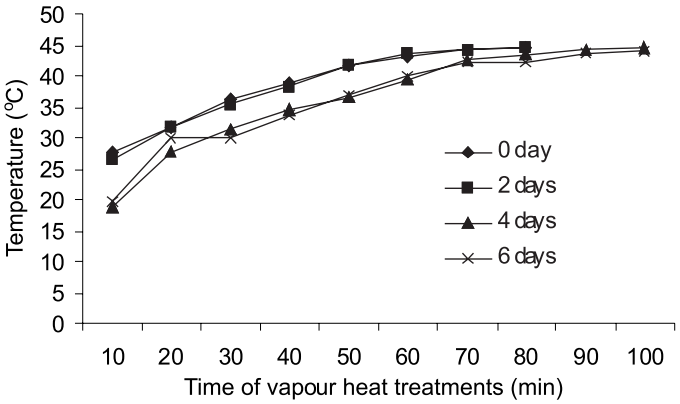

Fig. 1. Fruit centre temperature of mango fruits and air temperature during vapour heat treatment

length was observed at the 6th day. Ronald *et al.* (2007) found that the larva has three stages, and the third instar is about 2/5 inch long and similar results were recorded in this study. This result focused on the importance of identifying rearing time during which eggs change to pupae. The rearing time could attribute to positive effect on larval length because it could determine time for larvae development.

Heat tolerance test of oriental fruit fly in 'Tuu Shien' mango fruit: Time of VHT increased with the numbers of days following infestation. The values ranged from 70 to 100 min (0, 2, 4, and 6 days) for mango fruit centre temperature to arrive at 43°C (Fig. 1). At 0 d group, the treatment time was the shortest. But for the 6 d group, the treatment was prolonged to 70 min before mango fruit centre temperature reached 43°C. This proves that, total time of VHT is affected by ripening stage.

The alive instar percentage and mortality rate are shown in Table 3. There were significant differences in the alive instar rate between 15 and 30 min treatments. The mortality rates were increased when samples were treated with vapour heat for a longer time, except that the egg stage for 30 min. At the egg stage, the alive instar percent was different between treatment times, while mortality rate was higher when fruit centre temperature was at 43°C (53.17 and 40.00% for egg stage at 15 min and 30 min, respectively). This was because the VHT at 43°C for 30 min, killed the eggs before they could develop, so the alive instar rates were low. Additionally, for 1st, 2nd, and 3rd instar stage, the alive instar rate was reduced from 37.74 to 10.31% when treatment time was 30 min. For example, the 1st instar with increase of time the mortality rate increased from 32.18 to 62.26%. The 3rd instar with increase of time the mortality rate also increased from 45.59 to 89.69%. On the other hand, all stages with VHT, the mortality rates were increased negative when fruit temperature was at 43°C for 15 min except that the mortality rate decreased positive from the egg stage to the 1st instar stage. The mortality rates were increased negative when fruit centre temperature was at 43°C for 30 min. The alive instar rate was the highest in the control (no VHT), 96.79%, and mortality rate was the lowest. The data showed that the egg stage was the most tolerant to heat (Table 3).

Table 3. Heat tolerance test of oriental fruit fly in 'Tuu Shien' mango fruit

Stage	Treatments	Alive instar rate (%)	Mortality rate (%)
Egg (24 hrs)[y]	43°C, 15 min	46.83ab[x]	53.17ab
	43°C, 30 min	60.00ab	40.00ab
1st-instar (2 days)	43°C, 15 min	67.82ab	32.18ab
	43°C, 30 min	37.74ab	62.26ab
2rd-instar (4 days)	43°C, 15 min	64.66ab	35.34ab
	43°C, 30 min	33.30ab	66.70ab
3nd-instar (6 days)	43°C, 15 min	54.41ab	45.59ab
	43°C, 30 min	10.31b	89.69a
6 days	Control[z]	96.79a	3.21b
Mean		52.43	47.57
F-value		*	*
CV (%)		69.87	77.01

[x]Values in columns followed by the same letter are not significantly different according to the Tukey test of transformed data. F-value for main effect or interaction significant at $P>0.05$. z: no heat treatment.
[y]: The number in parenthesis is rearing time.

Vapour heat has been used worldwide to disinfest mangoes from fruit flies (Gaffney *et al.*, 1990). Sein (1935) reported that all immature fruit flies were killed in mangoes from Puerto Rico after exposure to vapour at 43°C for 4 h. Vapour heat was used by Koidsumi (1937) to disinfest mangoes in Taiwan. Sunagawa *et al.* (1987) reported that immature melon fruit fly, *Bactrocera cucurbitae* (Coquillett), in mangoes in Okinawa were killed with vapour heat at 44 ± 0.3°C, >90% RH when the pulp centre reached 43°C and remained at that temperature for 3 h.

Animal and Plant Health Inspection Service (1992) reported that vapour heat treatment at 43°C for 6 h was used on mangoes exported from Mexico. In addition, mangoes that were to be imported from the Philippines or Thailand to Japan had to be treated for fruit flies using vapour heat until fruit centre temperatures were 46.0°C (Philippines) or 46.5°C (Thailand) and held at the respective temperature for 10 min (Anonymous, 1975, 1987).

The small-scale disinfestations test of oriental fruit fly: One hundred oriental fruit fly eggs were inoculated per fruit and one hundred eighty mango fruits were used in small-scale test. Table 4 shows that the larvae survival rate after vapour heat treatments where the fruit centre temperature was 46.5°C for different time

Table 4. The small-scale disinfestation of oriental fruit fly in mango fruits

Treatments	Hatching rate (%)	Survival rate (%)	Mortality rate (%)
Control[y]	100.00a[x]	83.43a	16.56c
46.5° C, 10 min	100.00a	64.80b	35.20b
46.5° C, 20 min	0.00b	0.00b	100.00a
46.5° C, 30 min	0.067b	0.00b	100.00a
46.5° C, 40 min	0.00b	0.00b	100.00a
46.5° C, 50 min	0.00b	0.00b	100.00a
Mean	33.34	24.70	75.29
F-value	***	***	***
CV (%)	0.45	93.89	30.80

[x]Values in columns followed by the same letter are not significantly different according to the Tukey test of transformed data. F-value for main effect or interaction significant at $P< 0.001$. y: no heat treatment

intervals. There were significant differences in hatching, survival, and mortality rate between the treatments. After eggs inoculation, mangoes were kept for exactly one day at 25 ± 2°C; they were treated with vapour heat; then returned to room temperature for 7 days.

In the control (untreated with vapour heat) the larvae survival rate was 83.43%, while VHT at 46.5°C for only 10 min had 64.80% surviving larvae, a mortality rate of 35.20%. However, VHT for 20 to 50 min left no survivors. It must be noted that there was no difference in VHT besides the time of exposure. However, two eggs did hatch into larva in the batch that underwent to 30 min VHT. The hatching rate was 0.067% in VHT at 46.5°C for 30 min. This demonstrates that VHT with a fruit centre temperature of 46.5°C killed eggs completely after 40 min. Therefore, the treatment of 46.5°C at 40 min can be used for quarantine heat treatment for 'Tuu Shien' mango fruit.

Vapour heat treatments have been reported for Taiwan mangoes which infested with melon fly can be disinfested with vapour heat at 47.5°C until the fruit centre temperature was >46.5°C for 45 min (Kuo *et al.*, 1987). Vapour heat was also approved by Japan in 1986 to allow the import of mangoes from the Philippines (Merino *et al.*, 1985). Currently, Japan requires mangoes from the Philippines and Thailand to be treated with vapour heat until temperature of fruit centre is 46 and 46.5°C, respectively, and held at the respective temperature for 10 min (Anonymous, 1975, 1987). Australia Quarantine and Inspection Service (AQIS) (2008b) reported approval of a vapour heat schedule against Queensland fruit fly (*Bactrocera tryoni*) of 47°C for 15 min, in 'Kensington Pride', 'R2E2', 'Keitt', 'Palmer' and 'Kent' from Australia bound for the Japanese market. Additionally, vapour heat treatment was approved as quarantine treatment for *Anastrepha* species in 'Manila'; oriental fruit fly from Taiwan; and for Mexican fruit fly [(*Anastrepha ludens* (Loew)]. AQIS (2008a) also showed that mangoes from Taiwan imported into Australia must be treated until the pulp temperature has been held at 46.5°C for 30 min.

The large-scale disinfestation test of oriental fruit fly: Probit analysis was used to examine the results of the small-scale disinfestation test of oriental fruit fly. As recorded, in the Table 4, there was a mortality rate of 99.93% (data not shown) after 30 min VHT. However, this is unacceptable under probit 9, whose security requires a mortality rate of 99.9968%. Therefore, 40 min VHT was chosen for large-scale disinfestation tests. The results in Table 5 show that there were no survivors when fruit centre temperature was 46.5°C for 40 min. So, the mortality rate for oriental fruit fly eggs was 100%. This documented that, with VHT for fruit centre temperature exposed at 46.5°C for 40 min was sufficient to kill eggs in mango fruit.

Effect of vapour heat treatment on colour, firmness and total soluble solid of 'Tuu Shien' mango fruits: The colour of the skin of whole mango fruit was estimated and measured before and after VHT by optic subjective at three different times (0, 3d, 6d). Compared to the control, heat-treated fruit had higher value on the index, indicating yellowing of the skin as previously reported by Segarra-Carmona *et al.* (1990).

The colour by optic subjective for VHT treatments was significantly higher than for the control. These results emphasized

Table 5. Large-scale disinfestation of oriental fruit fly in mango fruits

Treatments	Number of tested eggs	Number of survivor	Mortality rate (%)
Control (without heat treatment)	6.000	1.511	32.68
46.5°C, 40 min	60.000	0	100

Table 6. Effect of vapour heat treatment (VHT) on colour, firmness, and total soluble solid (TSS) of mango fruits

Treatments	Colour	Firmness (N)	TSS (%)
At treatment	1.8b[x]	27.3a	16.3a
CK-3d	2.1ab	17.6bc	14.5ab
CK-6d	2.6ab	13.6c	14.4ab
VHT-3d	2.8ab	21.4ab	14.5ab
VHT-6d	3.2a	12.6c	13.7b
Mean	2.5	18.50	14.69
F-value	*	***	*
CV (%)	40.22	28.85	11.23

[x]Values in columns followed by the same letter are not significantly different according to the Tukey test of transformed data. F-value for main effect or interaction significant at $0.01<P<0.05$ (*), or $P=0.001$ (***). CK: No heat treatment. VHT: Vapour heat treatment

the influence of the duration of treatment on the VHT (Table 6). For each treatment, the colour value increased during storage time. Table 6 shows that after 6 d, all the treatments showed a rise in colour from 2.6 to 3.2. This was within the threshold 50% yellow and 50% green of the colour index. It was determined, that with a 3 d VHT treatment, the colour of mango fruit was maintained significantly better than with the other treatments. In addition, firmness value decreased during storage for all conditions except for VHT-3 d (Table 6). These results suggest that VHT-3 d maintained the best quality of mango fruit. Total soluble solid percent (TSS %) (Table 6) for CK and VHT was similar whatever the treatment conditions. However, there was a slight decrease of TSS which did not match characteristics of the normal ripening process. The VHT also induced a slight decrease of TSS until after day 6. Compared with the control treatments, VHT-6d treatment decreased the TSS% of mangoes. However, the post treatment changes in TSS% did not appreciably affect the quality of mangoes up to 6 days.

Sein (1935) reported that using VHT to prevent West Indian fruit fly infestation during refrigerated storage for 8 h at 43°C in a circulating atmosphere saturated with moisture did not alter the flavour, texture, and storage quality of 'White' mangoes. Furthermore, vapour heat at 43.5 ± 0.5°C for 3 h for melon fly disinfestations did not injure the mangoes (Sunagawa *et al.,* 1987). The susceptibility of the fruit to storage decay was reported by Hallman *et al.* (1990) in which vapour heat at 46-46.4°C for 3 hours and 45 min resulted in darkening of the oil glands in the peel of 'Marsh' grapefruit. Vapour heat treatment for quarantine security against Caribbean fruit fly at 43.5°C for 260 min on 'Marsh' and 'Ruby' grapefruits did not develop symptoms of quality deterioration (Miller *et al.*, 1991).

Hundred percent of eggs inoculated in 'Tuu Shien' mango fruits were killed with vapour heat treatment at 46.5°C for 40 min. Under this condition, no heat injury occurred and there was no affect on quality. Overall, vapour heat treatment may be used

as a quarantine and disinfestation technique for 'Tuu Shien' mangoes.

Acknowledgments

We thank Miss Ming-Hui Wang, Mrs Chun-Fen Lin (Department of Horticulture and Taichung Quarantine Center, Taiwan, respectively) for assistance with rear work for preparing fruit fly eggs. We are grateful to Prof. Adel A. Kader (University of California, U.S.A), Prof. Zora Singh (Curtin University, Australia) for their critical reading and valuable comments in improving the contents of the paper.

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Received: May, 2010; Revised: September, 2010; Accepted: October, 2010

Journal of Applied Horticulture, 12(2): 113-121, July-December, 2010

A world of flowers: Dutch flower auctions and the market for cut flowers

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Abstract

This paper gives an overview of international flower production, consumption and trade, focusing on the Dutch flower auctions in Aalsmeer, the world's leading flower trading centre. Data on prices and traded volumes for three important species of cut flowers (roses, chrysanthemums and carnations) for the period 1993–2008 are analyzed. Flower prices and traded volumes are extremely volatile. Although part of this volatility is predictable, because of regular seasonal variations in demand, a large proportion of the observed volatility is due to sudden shifts in supply. The real prices of cut flowers declined during this period, and there was a clear shift in consumer preferences toward roses and away from carnations. In addition, consumption of roses and carnations shifted from clearly seasonal toward more year-round consumption, while consumption of chrysanthemums followed consistent seasonal cycles throughout the period. During this period, non-European producers increased their market shares. This development can be traced to a significant decrease in cut flower prices relative to energy prices, especially after 2003.

Key words: Flower markets, flower production and trade, volatility, Dutch flower auctions, price analysis

Introduction

Cut flowers belong to a very special class of commodities. Flowers, like newspapers or fresh bread, are extremely perishable. Furthermore, the intrinsic value of flowers differs from that of most other commodities. While almost all agricultural commodities are produced and bought to satisfy nutritional or energy requirements, flowers are demanded solely to satisfy emotional needs. As such, flowers are in the same category as the arts, *e.g.*, a theatre performance or a music concert. Performing arts differ in that they may be stored as audiovisual recordings. Furthermore, flowers are bought to convey sentiments of different, sometimes completely opposite, types. Flowers are used both to signal sympathy in times of grief and as a token of joy and happiness. Bolle (2001) discusses such signals in the light of cooperation and exploitation, in terms of transaction cost economics. The combination of flowers' extreme perishability and their being demanded for multiple emotional and aesthetic reasons makes the market for cut flowers an interesting and challenging object for economic analysis.

The aim of this paper is to give an introduction to the international flower markets, with a focus on the Dutch flower auctions. First, we put flower prices in a historic perspective. The so-called 'tulip mania' in the 16th century is often referred to as history's first financial bubble. With the tulip mania as a historic backdrop, we move to the recent history of flower markets, presenting some vital statistics on production, exports, imports, consumption and prices since the 1990's. The two decades since 1990 represent the globalization of floriculture. Flower production requires labour and capital, in particular energy (heat), light (sun- or artificial light) and fertilizer. Energy comes as oil, gas or electricity, or alternatively as heat generated by the sun. The latter is more available in the southern countries, and increasing oil prices have gradually reduced the relative production costs of flowers in countries like Kenya and other African countries where major energy source is the solar energy. This process will be illuminated through some simple statistical relationships between flower prices and oil prices.

Flower prices: 500 years of roller coaster: The history of Holland as a flower-trading and flower-producing country dates back to the end of the 16th century. The history of the Dutch flower trade is discussed in, *e.g.*, van Lier (2005). In 1594, botanist Carolus Clusius (1526-1609) planted the first tulips in Dutch soil, only to see the whole collection stolen from the university garden that same year (van Lier, 2005). From then on, exotic plants were imported in increasing quantities from the Dutch East and West Indies to merchants in Amsterdam, who acted as suppliers to the great gardens of Europe. Some of the merchants also commissioned drawings and paintings of the flowers they had for sale, which were published in books. By 1630, dozens of books existed depicting flowers, especially tulips; these served as catalogs of the flowers for sale (van Lier, 2005).

The demand for tulips rose dramatically and between 1610 and 1637 the tulip trade developed into a so-called "fever", affecting the whole country. Garber (2000) gives an extensive analysis of the development, subsequently labeled "the tulipmania".

The mania soon reached the middle classes and, according to Mackay (1841), a popular tulip could cost as much as an Amsterdam townhouse. Why tulips only became the focus of a mania is hard to understand, as there were many flowers at the time that were considered more beautiful than the tulip. It has been suggested (*e.g.*, Garber, 2000) that the fact that the tulip was difficult to grow and susceptible to disease made its cultivation a challenge at which only the best succeeded (Pavord, 1999). In addition, some of the tulips developed striped flowers, where the pattern of stripes was unique for each bulb; this became the focus

of great attention. At that time, it was not known that the stripes were due to mosaic virus attacks (Lesnaw and Ghabrial, 2000).

What makes tulips different from most flowers is that they can be harvested and moved only between June and September; consequently, spot market trading could take place only in this period. During the rest of the year, futures contracts were made before a notary. In 1636, these contracts were formalized, but no deliveries were made, as the market collapsed in February 1637.

However, as a result of the tulip trade, the Dutch developed many of the techniques used in modern finance. In 1636, regular markets were opened in many Dutch cities. Foreigners entered the market and money flooded into Holland. Eventually, it became obvious that the capital inflow and rising prices would come to an end. Confidence vanished and panic spread. Prices fell abruptly and bulbs could not be sold at even a fraction of their previous value.

The price differences across the different bulb cultivars were huge. Therefore, Thompson (2007) developed a standardized, quality-weighted price index for tulip bulbs in the period from November 12, 1636, to May 1637. The bulbs were sold by weight, and prices were calculated as guilders per aas (aas = $1/564^{th}$ of an ounce). The calculation of the index is explained in detail in Thompson (2007). The price per aas increased from less than 10 guilders to approximately 200 in less than three months. From February 3 to February 9, 1637 (*i.e.* seven days), the price decreased by 50 guilders, and by the beginning of May 1637, the price had returned to the November 12 level.

According to Mackay (1841), several public meetings were held to try to pressure the government to bail out the unfortunate traders but without success. The problem ended up at the Provincial Council at The Hague, but a remedy was beyond the power of the government. The judges assumed this to be debt contracted in gambling, and therefore not debts in law.

So, according to Mackay (1841), the story ended. The final buyers had to carry their losses as best they could, and those who had gained from the high prices were allowed to keep their profit. The Dutch flower business suffered a severe shock, and it took years to reestablish confidence.

Until the 1980s, Mackay's presentation of the tulipmania, or "bubble", went unchallenged and mostly unexamined. More recent studies suggest that Mackay's research was incomplete and inaccurate. Goldgar (2007) argues that the tulipmania phenomenon was far more limited than previously thought, that only a handful of people experienced severe economic problems in this period, and that even for these people it could not be proven that the problems were due to the tulip trade. Even if prices had increased enormously, money had not changed hands. Therefore, profits were not realized and, unless they had made other deals on credit, the price collapse did not incur losses to traders.

Garber (1989) claims that one reason for the extreme price increase at the end of 1636 was that the bulbs had already been planted by then, which meant that the producers could not increase production as a response to the price increase.

Thompson (2007) argues that Garber's model cannot explain the abrupt price decrease. He believes that the dramatic price movements can be explained by changes in laws related to the futures contracts. According to Thompson, the essence of these changes was that futures contracts written after November 30, 1636, were to be interpreted as options. This meant that whereas the buyers were previously legally obliged to buy the bulbs, they could now choose to compensate the sellers with a fixed small percentage of the contract price (Thompson, 2007). Thompson argues that the mania was a rational response to legal changes. In any case, the tulipmania is still seen by many as a large economic bubble.

In any case, the early experience with tulip trading laid the foundation for elaborate and advanced trading institutions and pricing mechanisms in the flower business, notably the Dutch flower auctions.

Recent history of the world market for cut flowers: As recently as 40-50 years ago, the demand for cut flowers and potted plants around the world was generally satisfied by local production. In Europe, growing per capita income caused increased demand for flowers for everyday use and as gifts for special occasions. As transportation systems improved, more flowers were shipped from southern to northern Europe and the size of the European trade grew considerably. This was the start of the commercial flower industry as we know it today (Wernett, 1998).

The energy crisis in 1973 strengthened the comparative advantage of flower producers in southern Europe because of the large energy costs of greenhouse flower production. Energy costs constitute approximately 30-40 percent of the total variable costs in cut flower production in northern Europe, and significantly less in southern Europe. Increasing amounts of flowers from the south of Europe were therefore moved to the Dutch flower exchanges to meet the demand after 1973.

Later, increasingly, flowers bought in Europe were produced by Israeli producers. In Israel, flowers may be grown outdoors or in plastic tunnels all year round, eliminating both the energy costs and the fixed greenhouse costs that the European producers face. The Israelis faced two other limiting factors, however: transportation costs to Europe and water supply. These limitations were reduced through transport subsidies and research into watering systems to reduce water consumption in agricultural production (Wernett, 1998).

Starting in the 1970s, big marketing campaigns financed by the Holland Flower Council started to influence consumption patterns outside of Europe, and cut flowers from the Dutch flower exchange entered the American market, mostly through New York. At the same time, Miami developed as a base for flower imports from Colombia, for onward distribution in the USA. This led to strong competition for local American producers that the Europeans used to their advantage. South American producers bought plant varieties from Europe, and North American producers were persuaded to buy production systems from Europe in order to counter the competition from the south (Wernett, 1998).

During the 1990s, African countries, in particular Kenya, exported increasing quantities of cut flowers to the European market. Together with the Israeli flower industry, Kenya is now a major competitor to the European producers.

As African producers entered the European market, European flower traders started to expand into Asia, especially to Japan,

exporting cut flowers as well as production systems and technology. This drive into Asia was helped by aggressive marketing campaigns. Commercial flower production in Asia started to develop because of increasing demand for low-priced flowers from the European market and European, mainly Dutch, producers started to produce in East Asian countries.

What makes flower production in Asia different to that in Africa and South America is that the latter produce flowers almost exclusively for export, whereas in Southeast Asia there is a growing market for local consumption because of growing incomes.

In the future, the largest potential for development and expansion of the flower industry is assumed to be in Asia, both for local consumption and for export. An in-depth analysis of the history of flower markets and the potential of Asian commercial flower production has been made by Wernett (1998).

Flowers by numbers - *International production and trade*

In 2008, the total area used for cut flowers and potted plants in the world was approximately 532,000 ha, an increase of 33 percent from 2005. The biggest producers in terms of land use were China with 286,000 ha (2006) and India with 70,000 ha (data from 1999 only). China almost doubled its flower production acreage during the last three years of the study period; the same is probably true for India. Almost 75 percent of all flower production land was in Asia, a 12 percent increase during the last three years. South America had almost the same area as Europe, approximately 50,000 ha, both stable since 2005. The data included on flower production, exports, imports and consumption are collected from International Statistics Flowers and Plants, 2005 and 2008.

If we look at the value of production, the picture is somewhat different. The total value of the world's flower production was approximately €24 billion in 2008, a 33 percent increase from 2005. European production constitutes almost half that value; the value of Asian production is approximately €7 billion.

The total value of world imports of cut flowers and potted plants in 2007 was estimated at €10.3 billion, Germany being the single biggest importing country with €1.5 billion (the EU data for 2007 include data for two new member countries, Bulgaria and Romania). By comparison, USA and Japan imported flowers for €893 million and €241 million, respectively (International Statistics Flowers and Plants, 2005 and 2008).

The total value of flower exports in 2007 was €10.9 billion, of which the Netherlands was responsible for almost half. European exports constituted approximately two-thirds of total exports. The Americans were the second biggest exporters with €1.8 billion, (with Colombia, Canada and Ecuador as the biggest exporting countries). Asia was exporting approximately €1 billion and Africa €820 million. Kenya was the biggest flower exporting country in Africa with €500 million, up approximately 100 percent from 2004.

Table 1. Value (€ 1000) of imported cut flowers from Africa, Latin America, Asia and the Middle East to the Netherlands and EU (total).

Exporting county	The Netherlands		EU total	
	2004	2007	2004	2007
Africa, total	288,806	312,365	347,569	447,371
Kenya	144,226	205,029	235,378	312,703
Latin America, total	64,844	105,615	171,934	235,533
Colombia	18,268	27,274	84,297	115,586
Ecuador	42,648	72,158	79,167	110,421
Asia (Middle East excluded)	4,546	5,394	21,490	26,574
Middle East, total	65,574	46,961	101,225	91,015
Israel	60,713	40,942	85,510	73,989
Total	423,770	470,335	642,218	800,493

Table 1 shows the value of imports from the major non-European flower producers into the Netherlands and the EU. More than half of the imports in 2007 came from Africa, with Kenya as the dominant exporting country. Almost 40 percent of total EU imports came from Kenya and together with Israel, Colombia and Ecuador these countries supplied 77 percent of EU imports (€613 million out of approximately €800 million in 2007). Total imports to Europe from non-European countries increased by 25 percent from 2004 to 2007, and the imports from Kenya by 75 percent in the same period. More than half of Europe's flower imports went through the Netherlands (in 2007). This amount increased by approximately 60 percent during the 10 years to 2007. In 2007, Great Britain and Germany imported flowers valued at approximately €170 million and €50 million, respectively, from non-European countries.

There is also a significant intra-European flower trade with the Netherlands as the focal point. Almost half of Germany's imports, more than 60 percent of Great Britain's imports and roughly 40 percent of the flower imports to France, by value, come from the Netherlands.

Fewer than 10 species make up the bulk of the cut flower trade: roses, chrysanthemums, tulips, lilies, gerberas, cymbidium, freesias, anthurium and alstromeria. While the value of cut flower species traded at Dutch auctions increased by 25 percent during the period 1998-2008, the value of the rose trade in the same period increased by more than 70 percent.

Table 2. Per capita consumption (€) and market value of consumption (million €) of flowers, 2006

Country	Per capita consumption			Population	Estimated market value		
	Cut flowers €	Plants €	Flowers (total) €	(Million)	Cut flowers (€ million)	Plants (€ million)	Flowers, total (€ million)
Germany	36	48	84	83	2,988	3,984	6,972
Netherlands	54	32	86	16	864	512	1,376
Norway	62	62	124	5	310	310	620
Russia	5	1	6	143	715	143	423
Switzerland	82	43	125	7	574	301	875
Europe	23	16	38	680	15,755	10,740	26,060
Japan*	54		54	128	6,912		6,912
USA*	21		21	306	6,426		6,426

*Cut flowers only

Table 2 shows the consumption of flowers (cut flowers and total) per capita in 2006, as well as the value of consumption. When it comes to total demand for flowers, Switzerland and Norway had the highest per capita total consumption of flowers in the world. The average per capita consumption of cut flowers (in 2006) in Europe (€23), even including the relatively low consumption in Eastern Europe and Russia, is higher than the per capita consumption in the USA (€21), but considerably lower than in Japan (€54). When we take into account the population of the different countries, Germany is by far the biggest consumer in Europe with a total consumption of flowers and plants of almost €7 billion. Of this, the value of cut flower consumption is €3 billion, which is approximately half the value of cut flower consumption in the USA. Japan is the biggest cut-flower-consuming country in the world with a value of €6.9 billion.

The Dutch flower auctions: The history of today's Dutch flower auctions dates back to 1911-12, when flower producers in the city of Aalsmeer established two flower auctions: "Bloemenlust" on the east side and "Central Aalsmeer Auction" in the city centre. The auctions were established because producers felt they were in the hands of agents who manipulated prices and that the agents were not always reliable payers (van Lier, 2005).

The concept of the cooperative auctions was adopted from the fruit and vegetable industry. The producers hoped they would collectively become stronger and, by offering their product exclusively at the auctions, they forced the buyers to trade through the so-called auction clock. On a "one-armed clock", the clock arm moves counterclockwise, starting at a high price, which falls until the first buyer stops the clock at the price he or she is willing to pay. Thus, the introduction of the auctions seemed to shift power from agents to growers.

The aim of the clock auction was to generate a fair price. It increased competition on the demand side, because the buyers could get information about the prices and quantities of their competitors. On the supply side, it led to higher quality of the flowers offered at the auctions.

In 1972, Bloemenveiling Aalsmeer was established through the merger of several smaller auctions; most recently, in 2007, Bloemenveiling Aalsmeer and FloraHolland, the two largest flower auctions in the world, merged. The merged company, called FloraHolland, started its operations in January 2008.

The main reason given for this merger was the threat from developments in the international flower market, especially the opening of a flower market in Mumbai, India, and another one in Dubai. As India has evolved to be a very big flower producer, as well as a substantial consumer, and as Dubai is closer to the African flower producers than the Netherlands, there was a fear in Aalsmeer that trade would shift toward Dubai.

The Dutch flower auctions have so far managed to develop and sustain a leading position in traded volume as well as in research, production, marketing, standardization, information and education (Wernett, 1998). In 2008, the merged FloraHolland had a turnover of €4.07 billion.

The flower auction in Aalsmeer is today one of Floraholland's six auction sites in the Netherlands but, because of its history and size, Aalsmeer requires some special attention. In 2008, Aalsmeer had a clock turnover exceeding 11 billion cut flowers and 800 million plants, amounting to a turnover of some €2.4 billion, more than half of the total clock turnover of Floraholland. The auctions take place in a huge trade centre covering approximately 1 million square meters, which is roughly comparable to 250 soccer fields. Within this trade centre, very complex logistical processes and auctions take place, which in turn determine world prices for flowers.

In any given week, around 100 species of cut flowers are traded in Aalsmeer and for many of the species there are several varieties. As many as 30 to 40 different varieties of roses are traded, with each variety possibly having different colours and lengths. There are also quality differences. Therefore, in contrast to many agricultural and industry products, fresh flowers cannot be treated as a well-defined, homogeneous product. Cut flowers are very fragile, they cannot be stored, the supply is relatively unpredictable and price variations over time and among cultivars are substantial. Trip *et al.* (2000) examined the price-predicting abilities of Dutch chrysanthemum farmers, finding evidence that predicting relative price positions (relative to other cultivars) was a skill. They also found that price differences among cultivars were nonrandom in time and that growers could adapt their production planning and cultivar choice to benefit from expected price variations.

Approximately 9,000 individual producers market their flowers at the auctions of FloraHolland, of whom 5,000 are exchange members. Since 2007, producers from non-European countries can become members of the cooperative. The new members are mostly "off-shore" Dutch producers located in Kenya and Uganda as well as Israeli growers. Each member has to make a deposit to the cooperative equal to 1 percent of their sales. The cooperative pays interest to members and the deposit is fully returned after nine years. Members can also give interest-bearing loans to the cooperative. The general assembly meets twice a year and members' voting power is determined by their sales (deposit).

One important objective of the FloraHolland cooperative is to sustain and improve its market position by offering quantity, quality and variety. The declared objective of FloraHolland, a nonprofit service organization, is to offer their members the best sales possibilities at a low cost (FloraHolland, undated).

The auctions: The day starts early at the Dutch flower auctions. The night before each trading day (Monday-Friday), flowers are unloaded from numerous trucks at the auction halls. The cut flowers are stored in carts in cold rooms. At 4:30 a.m., the flowers are transported to the huge collection halls and sorted by species and quality.

Each unit is quality checked and given a unique number. Then the carts are connected to each other and dragged into the auction rooms on small electrical trains. The auctions start at exactly 6:30 a.m.

As mentioned above, the auction mechanism is the so-called Dutch auction. As opposed to an English auction, the starting price is high rather than low. The auctioneer announces the flowers to be sold, including batch size, minimum buying quantity, name of the producer and comments, if any, from the quality inspector.

The bidding is controlled by a huge clock-like screen indicating

the unit price (*e.g.*, €100, €10 or €1). A blinking light on the screen marks the starting price, which then moves downward on the clock. A buyer will press the button at his or her desk in the auction room to stop the clock when the light hits the price he or she is willing to pay.

When a buyer stops the clock, he or she must immediately communicate to the auctioneer the quantity purchased at the given price. Soon afterward, the clock moves to a slightly higher price before it again starts its downward move. This procedure is repeated until the whole batch is sold. The procedure then re-starts for the next batch of flowers to be auctioned. Each unit of flowers has a minimum price. If the minimum price is not achieved, the whole batch is withdrawn and destroyed immediately after the auction.

Thus, during the auction, each of the bidders must choose a reservation price, which is where the bidder would stop the clock if the price should fall to that level without exhausting the offering. The bidder with the highest reservation price wins the object at his or her chosen price. This type of auction is often described as an "open first-price auction" and a more precise definition is "A sequential, private value auction of identical objects" (van den Berg *et al.*, 1999). It is considered strategically equivalent to a "first-price sealed-bid auction". There is a huge body of literature on auction theory (set ups, outcomes and so on). A classical reference on auctions and bidding is McAfee and McMillan (1987). Van den Berg *et al.* (1999) analyzed the presence of declining prices at the auctioning of roses at the Dutch flower auctions. In addition, Kambil and van Heck (1995) performed an in-depth study of the features, strengths and weaknesses of the Dutch auctions and the effects of the introduction of new trading mechanisms based on information technology. Usually, there are only data on winning bids, but van den Berg and van der Klauuw (2007) performed an interesting structural empirical analysis of the auctions of potted plants using data on losing bids.

The buyers at the auctions mostly represent large flower wholesalers, exporters and large retailers. Up to 90 percent of flowers sold reach their final destination within 24 hours. Transportation within Europe mostly takes place in cooled trucks. Flowers are sent to the USA by plane; they usually reach New York during the evening or night of the sales day, and wholesalers in the New York flower district receive them as early as 3:30 a.m.

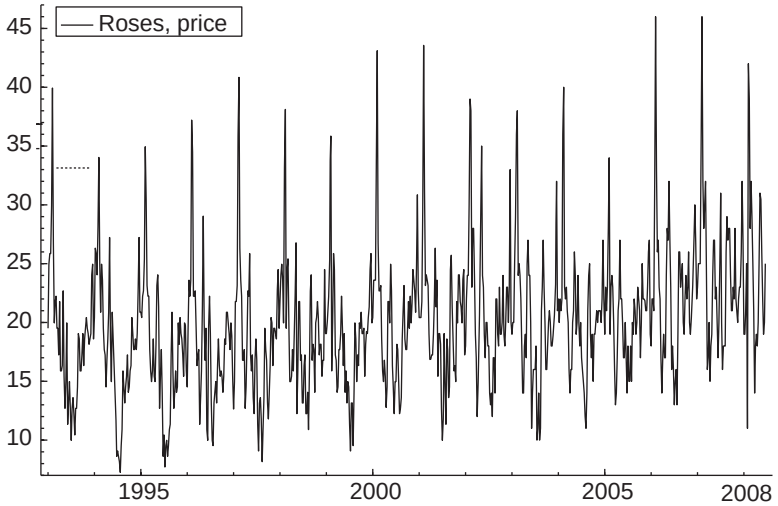

Fig. 1. The nominal price of roses (Eurocent per stem) week 1, 1993 to week 25, 2008.

The 39 auction clocks of Floraholland are at the heart of the auction system. Every sales day, roughly 1,000 buyers gather in front of the clocks to follow the prices of the different flowers for sale. Different products are offered at different clocks. Each transaction takes only a few seconds. The auctions are therefore carried out at a tremendous speed, which is important for a highly perishable product. The FloraHolland auctions have approximately 125,000 transactions per day, which amounts to more than 12 billion cut flowers and more than 800 million potted plants traded each year (Floraholland, undated).

More than 60 percent of the world flower trade goes through the Dutch auctions. It is also possible to trade at the auctions without being physically present, following the clock via the Internet. There is also a gradual transition toward the flowers being presented through pictures rather than live at the auction, so that the flowers do not have to leave the cooled storage until they are transported directly to the buyer.

Floraholland employs 4,500 people, 2,000 of whom are in Aalsmeer. A further 12,000 people (in Aalsmeer) are employed in supporting activities such as wholesaling and exporting. The flower sector in the Netherlands is a significant sector, economically and socially. The contribution of the Dutch flower trade to the balance of trade is 20 percent. The direct and indirect employment in the flower sector is approximately 250,000 full-time jobs (Floraholland, undated).

Prices, price volatility and turnover at the Dutch flower auctions 1993-2008

Prices and traded volumes at the Dutch flower auctions are published weekly in "Vaakblaad vor der bloemisterij". Here, weekly data for the period January, 1993 to June, 2008 are analyzed.

Flower prices: Fig. 1 shows the weekly nominal rose prices, measured in Eurocent per stem, during the period 1993-2008. The rose price trended upward by 1.9 percent annually, as compared to the price of carnations, which increased by 1.2 percent annually. Chrysanthemums, however, saw stagnating prices during this period. The average inflation (CPI) in the Netherlands for this period was 2.3 percent annually, which means that the real price of cut flowers fell by 0.5-1 percent annually.

The demand for cut flowers is extremely seasonal, generating regular calendar patterns in prices. Therefore, to describe prices in a somewhat longer run, the series are smoothed (12-month moving average). Fig. 2 visualizes what can be labeled the business cycles in the flower trade.

Disregarding the sharp seasonal price movements, rose prices trended quite steadily upward, particularly after 2005. Chrysanthemums, with no long-term price increase, saw some large fluctuations with price peaks in 1998 and 2001. The long-term price growth for carnations is mainly a result of a price surge after 2000; at the end of the 1990s, carnation prices dropped dramatically.

Traded volumes: From Fig. 3, we can see the cycles and trends in traded volumes during the study period. For the auction as a whole, there was a growth of 1.1 percent on an

annual basis, mainly due to the increased demand for roses (+ 2.6 percent annually). For chrysanthemums, the traded volume during this period was stable; for carnations, there was a strong negative trend (12.4 percent on an annual basis).

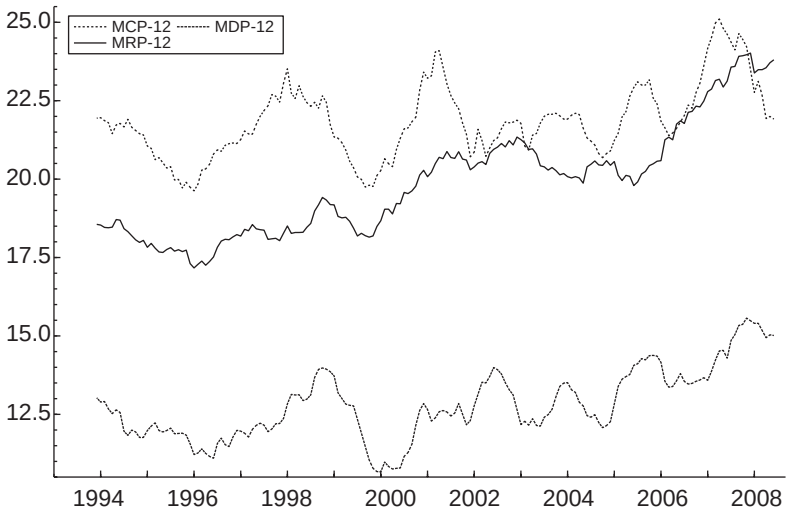

Fig. 2. Smoothed prices (12-month moving average) for roses (MRP-12), chrysanthemums (MCP-12) and carnations (MDP-12); Eurocent/stem, 1994-2008

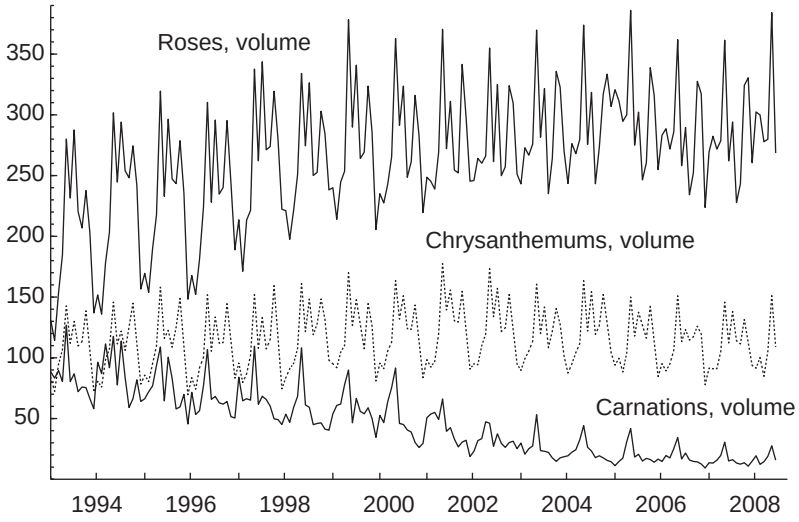

Fig. 3. Traded volumes (million stems) of cut flowers per month in the period January, 1993 - June, 2008.

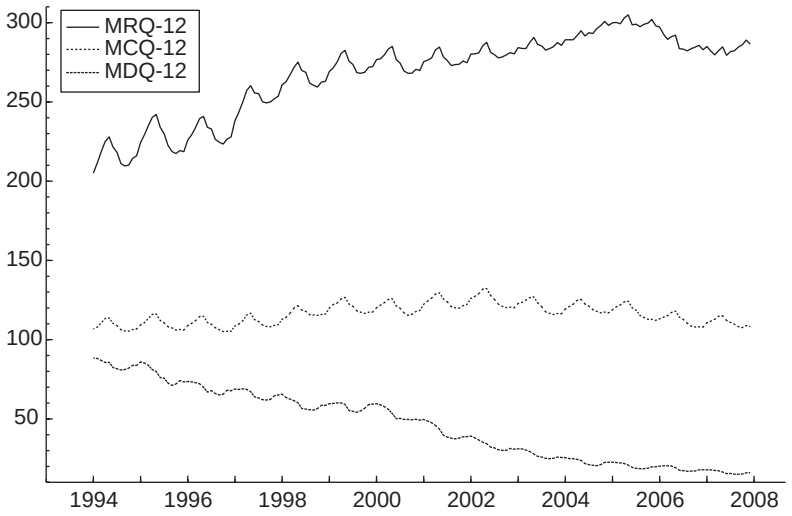

Fig. 4. Smoothed volumes (12-month moving averages) for roses (MRQ-12), chrysanthemums (MCQ-12) and carnations (MDQ-12); million stems, 1994-2008

The calendar patterns in prices are obviously reflected in volumes. Fig. 4 shows the smoothed (12-month average) volumes.

As can be seen, all three species had distinct calendar cycles up to 2000/2001. After that date, demand for roses and carnations appeared to be smoother, while chrysanthemums maintained strong seasonalities throughout the period. Thus, the consumption pattern for roses and carnations seems to have changed over time, toward a more year-round, or "everyday" consumption, while the demand for chrysanthemums is still quite traditional, linked to the time of the year and to events occurring each year.

Seasonalities in prices and volumes: The seasonal patterns are further illustrated in Fig. 5, displaying the mean prices for three main species of cut flowers over weeks 1-52. The overall mean prices of roses, chrysanthemums and carnations were approximately 20, 22 and 13 Eurocent/stem, respectively. Around these averages, the coefficients of variation (CV) were between 18 and 30 percent on a monthly basis, which makes flowers an extremely volatile commodity. The seasonal variation in prices was much higher for roses and chrysanthemums where the average price in the winter was as high as 2-2.5 times the average price in the middle of the summer.

Fig. 5 shows very strong seasonal cycles in the prices, but the cycles were not identical for the three groups of cut flowers shown. Roses were the most extreme, with a high of 39 Eurocent/stem before Valentine's Day down to 13 at the end of July. Again, chrysanthemums showed a similar pattern to roses. Chrysanthemums usually had a lower price than roses in weeks 14-38 and higher prices the rest of the year (with the exception of Valentine's Day sales).

Carnations had very different cycles to those of other cut flowers. Prices were relatively higher in February, June and October and lower in December and April. The differences between the high and low prices were smaller for carnations than for the other cut flowers.

Fig. 6 shows the demand cycles over the year for roses, chrysanthemums and carnations. For all species, traded volumes were relatively low during the winter period with the exception of sales around Christmas time. Thus, during the last couple of weeks of each year, traded volumes were up. For roses, January was a month with relatively low sales, but in February, there was a distinct peak, particularly in week 6, coinciding with Valentine's Day and then Mother's Day. The traded volume of roses increased steadily until it reached a maximum at the beginning of May. It then decreased slowly until July/August. After a slow increase in early fall, the sales decreased again until the beginning of December; finally, there was the Christmas sale.

Chrysanthemums followed roughly the same pattern as roses, but the peaks were less distinct. For cut flowers seen as a whole, the spring turnover was remarkably higher than the turnover during the rest of the year. This was mainly

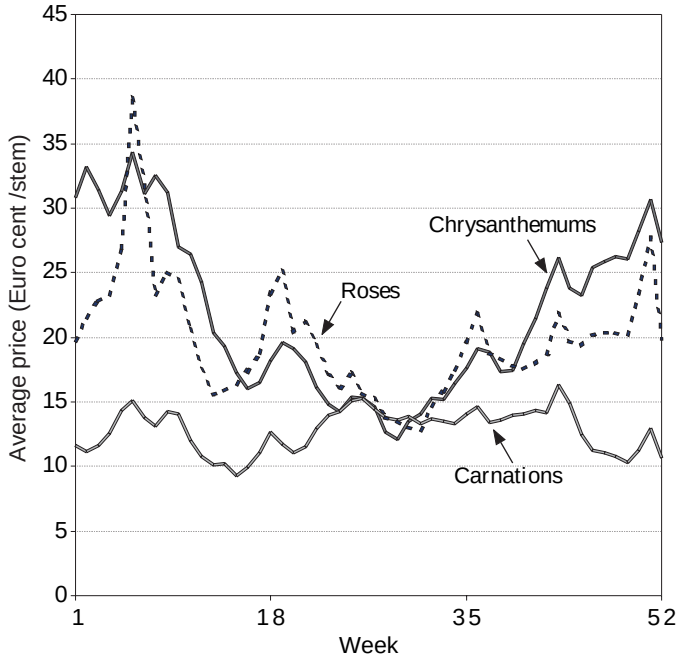

Fig. 5. Average prices (Eurocent/stem) of cut flowers each week, weeks 1-52, 1993-2008

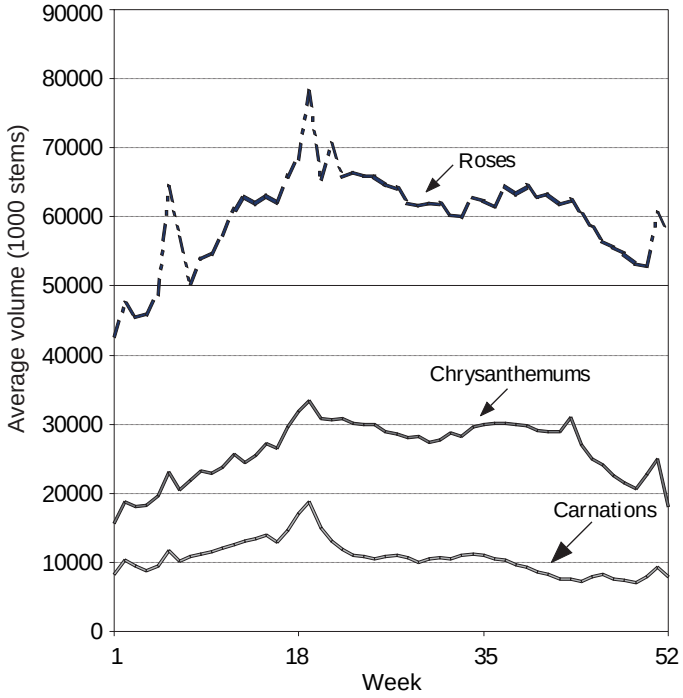

Fig. 6. Average weekly volume of different groups of cut flowers, week 1, 1993 – week 25, 2008

due to the demand for tulips and other bulbs in early spring.

Relative prices and consumer preferences: No big changes in relative production costs across different flower species occurred during the past 15-20 years. Hence, changes in relative prices may be interpreted as changes in consumer preferences.

Fig. 7 compares long-run changes in prices of different species using December 1993 as a common base. For most of the 1990s, prices tended to move together. Then, in 1998-99, a general reduction in prices took place, particularly for carnations. After that time, the rose price increased clearly more than the price of the two other species. While rose prices were up by almost 30 percent at the middle of 2008 compared with the 1993 level, carnations were up by only about 15 percent and chrysanthemums down by roughly 5 percent.

Fig. 8 illustrates this from a different angle, showing the scatter plot for the relative rose/chrysanthemum price together with a series for the smoothed average (exponentially weighted, alpha = 0.3) and the trend line. While a rose stem in the early 1990s was priced on average at 80-90 percent of a chrysanthemum stem, the rose stem attracted roughly the same price as a chrysanthemum after 2005. Thus, there seems to be a long-term trend in consumers' preferences toward roses relative to chrysanthemums. However, the huge and regular gyration in the relative price clearly show that the two species have their separate high weeks when the relative price may move by as much as 30-40 percent over very short periods.

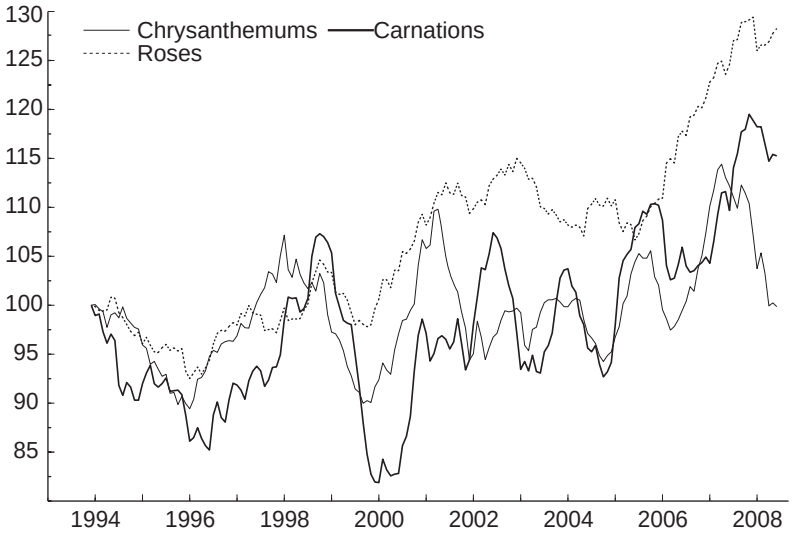

Fig. 7. Price indices (smoothed) for roses, chrysanthemums and carnations 1993-2008 (December 1993 = 100).

Roses saw a price increase relative to chrysanthemums during the study period. Disregarding the seasonal price variations, roses became roughly 20 percent more expensive during the period. Carnations also became more expensive than chrysanthemums in this period, by approximately 10 percent.

Changes in consumer preferences are also revealed through changes in realized demand. Figs 9 and 10 show the relative traded volumes of roses versus chrysanthemums and carnations, respectively.

Disregarding seasonal variation in demand, the volume of roses traded in 1993 was about twice that of chrysanthemums. By the end of the period, in 2008, the volume of roses traded increased to more than 2.5 times that of chrysanthemums.

Roses clearly outpaced the other two species in terms of turnover and for carnations, even at an accelerating pace. For roses versus carnations, the change was extreme. At the beginning of the period, the volume of roses traded was about twice that of carnations, while at the end of the period (2008), the volume of roses traded was more than 20 times that of carnations. Therefore, rather than a linear trend as in roses versus chrysanthemums, we observed an exponential trend.

Flower prices, energy prices and the international flower trade: Cut flowers are beautified energy. During

photosynthesis, carbon dioxide and water, in the presence of light and energy, are transformed into organic material and oxygen.

Energy costs form a large proportion of the variable costs of floriculture in Holland, as in other North European countries. The Dutch greenhouse industry accounts for 7 percent of the total energy use in the Netherlands, and approximately 4 percent of total CO_2 emissions (Lansink *et al.*, 2001).

Energy costs can be reduced by investments in energy-saving technologies. The Dutch greenhouse sector has signed an agreement with the government aiming to reduce the energy use per unit of production by 65 percent between 1980 and 2010 (Stuurgroep Landbouw en Milieu, 2000). Energy use has been reduced since 1980 but, according to Stuurgroep Landbouw en Milieu (2000), it will be very difficult to achieve that target.

Net present value calculations evaluating the profitability of investments in energy-saving technologies in Dutch floriculture predict a much higher rate of adoption of such technologies than is actually observed (Diederen *et al.*, 2003). One possible explanation for this is that the profitability of the investment is uncertain because of the stochastic nature of energy prices (Hasset and Metcalf, 1993). There is also uncertainty about the effects of increased production in other countries, *viz.* in Africa.

Another way to reduce energy use in the floriculture sector is to substitute solar power for oil, gas and electricity through imports from flower producing countries better endowed with sunlight and not using much oil, gas and electricity for flower production.

If we observe large reductions in the long-term flower price/energy price ratio, this can be interpreted as the effect of changing production location, *i.e.*, imports from countries in Africa, South America and Southeast Asia.

Fig. 11 shows the ratio of monthly rose (Eurocent/stem) to crude oil (USD/bbl) prices. The Fig. shows that until 1998 this ratio fluctuated around 1. From 2002 to 2003, this ratio decreased dramatically, and by 2008 it had fallen to approximately 0.25. Other cut flower prices show the same trend. We also observed less seasonal variation in the flower-oil price ratio. In other words, the price of cut flowers decreased dramatically relative to the price of energy (oil) after the 1990s. There has been a indirect change in use of energy sources in flower production and through increased imports, solar power has substituted for oil and gas requirements.

As shown in Table 1, from 2004 to 2007 there was 25 percent increase of imports from non-European (African, Southeast Asian and South American) countries to Europe. Rose imports from these countries to the EU countries increased by 46 percent in the period 2004-2007. This clearly supports the hypothesis that changes in the output price/energy price ratio can be used to explain shifts in location of flower production. This is not the focus of this paper, but would be an interesting extension of this work.

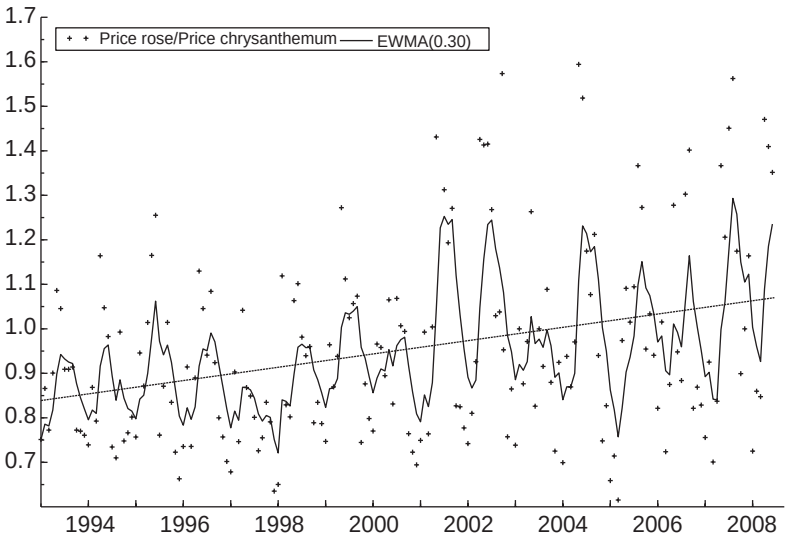

Fig. 8. Relative prices roses/chrysanthemums, January 1993 to June 2008 (monthly data). Scatter plot; trend and smoothed (exponentially weighted moving average, alpha = 0.3).

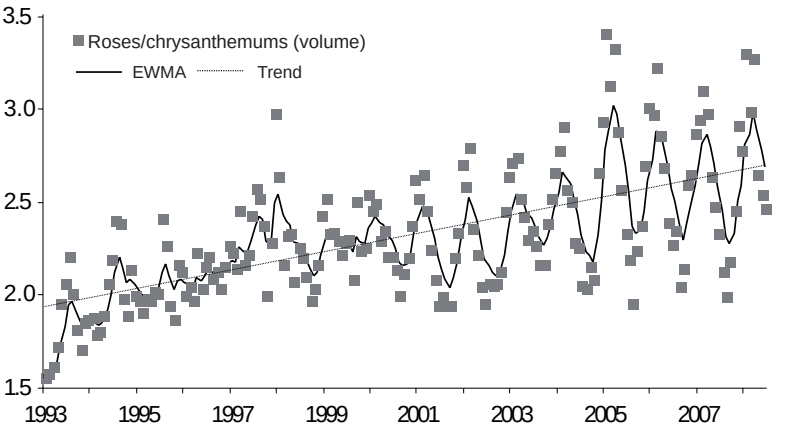

Fig. 9. Relative traded volumes of roses/chrysanthemums from January 1993 to June 2008 (monthly data), scatter plot, trend and smoothed (exponentially weighted moving average, alpha = 0.3).

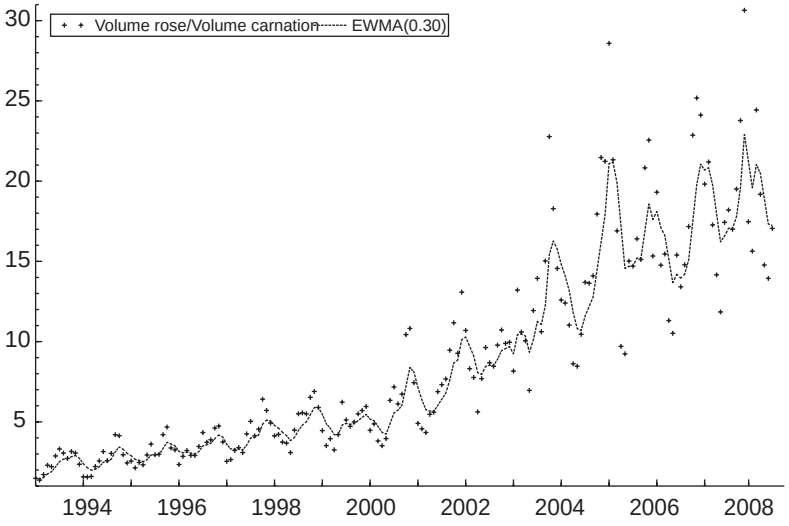

Fig. 10. Relative traded volumes roses/carnations from January 1993 to June 2008 (monthly data), scatter plot, smoothed (exponentially weighted moving average, alpha = 0.3).

Prices and traded volumes at the Dutch flower auctions during the 15 years from 1993 to 2008 reveal a number of distinct patterns and trends. For

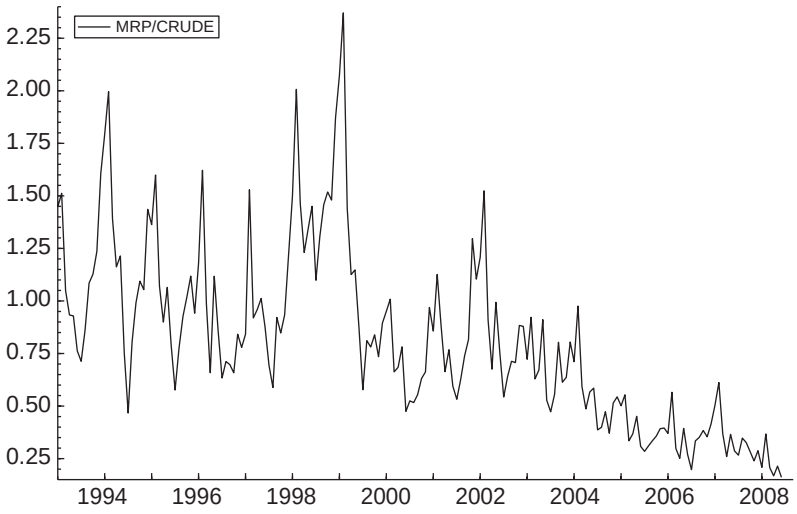

Fig. 11. The ratio of monthly rose prices (MRP) measured in Eurocent/stem relative to the price of crude oil measured in USD/bbl, from January 1993 to June 2008.

one, prices are highly volatile with persistent and strong seasonal patterns. The seasons are largely unique to each species of flowers. However, for some species the seasonality has gradually become less distinct. This is particularly the case for roses, which now seem to be year-round flower, while the demand for chrysanthemums continued to follow a more traditional cycle during the period of investigation. Flowers have become less expensive in real terms since the 1990s. Further, a relative increase in the price and demand for roses compared to other cut flowers indicates shifts in consumer preferences toward roses. Roses are clearly outpacing the two other major species in terms of turnover during the period of investigation, and for carnations, this is happening at an accelerating pace. While production in Europe is stable or declining, it is increasing rapidly in Africa, Asia and South America, and many Asian countries have experienced strong growth in consumption. This shift can also be traced as a decrease in cut flower prices relative to energy prices, especially during the last five years of the study period, due to strong growth in exports of flowers from Africa, notably Kenya, to Europe.

Acknowledgments

The author thanks the referee, Dr. Dadi Már Kristófersson, for valuable comments on a previous version of the paper.

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Received: March, 2010; Revised: July, 2010;
Accepted: October, 2010

Journal of Applied Horticulture, 12(2): 122-124, July-December, 2010

Sex determination in *Pistacia* species using molecular markers

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Abstract

Sex identification in *Pistacia* species are economically desirable. Regarding long juvenile stage in *Pistacia* species and lack of morphological method to identify sex in this stage, molecular marker could facilitate breeding program. Aim of the study was to identify a marker, closely linked to sex locus in *Pistacia atlantica* Desf *mutica*, *P. khinjuk* and *P. vera* var. Sarakhs. For this purpose, samples were collected from male and female individual trees from each species and their band patterns were analysed according to band specific presence or absence. Twenty Random Amplified Polymorphic DNA (RAPD) primers and a pair Sequence Characterized Amplified Regions (SCAR) primer were tested to determine sex in wild *Pistacia* species. Among RAPD primers, only BC1200 amplified a specific sex band which was present in female plant. The results indicated that all individual samples amplified an approximately 300 base pairs fragment in female trees which was absent in male samples. Although sex determination mechanism in *Pistacia* is unknown, it might be controlled by single locus acting as a trigger. However, SCAR technique is a reliable technique to identify gender genotypes in seedling stage of *Pistacia* species, that would help to save time and expanses in breeding program.

Key words: *Pistcia* spp, sex identification, SCAR- PCR, juvenile stage.

Introduction

Pistachio belongs to *Anacardiaceae* family which includes 11 species (Zohary, 1952). *Pistacia atlantica* Desf. *mutica*, *P. khinjuk* and *P. vera* var. Sarakhs are three main wild *Pistacia* species in Iran, which are applied as rootstock for *P. vera* and oil extraction in some countries (Kafkas *et al.,* 2002). Dioecious plants are thought to be the most evolved members of the plant kingdom in terms of sex differentiation. Therefore, it is impossible to determine sex during the pre-reproductive phase unless a genetic sex marker is available for this purpose (Korpelainen *et al.,* 2008). In many dioecious plants, their economic value and breeding schemes for commercial use of genetically transformed materials are influenced by sex (Alstrom-Rapaport *et al.,* 1998). The sex of pistachio cannot be known until it reaches reproductive age (Hormaza *et al.,* 1994).

With regard to long juvenile stage in dioecious trees, molecular markers have been extensively used in dioecious plant breeding to save time and economic source (Jiang and Sink, 1997). Several researchers showed that random amplified polymorphic DNA (RAPD) banding patterns have been linked to sex in *Hippophae rhamnoides* (Persson and Nybom, 1998), *Salix viminalis* (Alstrom-Rapaport *et al.,* 1998), *Piper longum* (Banerjee *et al.,* 1999), *Silene latifolia* (Zhang *et al.,* 1998), *Pistacia vera* (Hormaza *et al.,* 1994), *Encephalartos natalensis* (Prakash and Van Staden, 2006), *Actinidia chinensis* (Harvey *et al.,* 1997) and *Ginkgo biloba* (Longdou *et al.,* 2006). Sequence characterized amplified regions (SCAR) markers have been widely used to identify between two sexes in *Asparagus* (Gao *et al.,* 2007), *Carica papaya* (Bedoya and Nunez, 2007), *Rumex nivalis* (Stehlik and Blattner, 2004).

In many dioecious plants, even when sex determination is regulated genetically, no heteromorphic sex chromosomes are found. Sex chromosomes were indistinguishable in *Pistacia* species. Matsunaga (2006) has reported, in such cases, because sex chromosome cannot be identified by their size or shape, they are identified by trisomic analysis or analysis of genetic marker.

Sex-linked genetic markers based selection is an appropriate technique in breeding programs and is useful for understanding of genetic map and dioecism in *Pistacia* species. It was identified that RAPD marker is linked to sex locus in *P. vera* (Hormaza *et al.,* 1994), *P. eurycarpa* and *P. atlantica* (Kafkas *et al.,* 2001). Then by converting the RAPD primer to SCAR and Touchdown-PCR technique, sexual genotype identified in *P. vera* (Yakubov *et al.,* 2004).

To our knowledge, no sequence information on the aspect of *Pistcia* species genome is available. There are only few reports on molecular sex markers for wild *Pistacia*, so the present study is the first report on the use SCAR technique for sex determination in *P. atlantca* Desf. *mutica*, *P. khinjuk* and *P. vera* var. Sarakhs.

Materials and methods

Plant material and genomic DNA isolation: Fresh leaf samples from *P. khinjuk*, *P. atlantica* Desf. *mutica* and *P. vera* var. Sarakhs trees were collected from Iran Pistachio Research Institute (IPRI) in Rafsanjan. The samples were quickly transported to laboratory

and kept at -80°C until use. Genomic DNA was extracted according to Doyle and Doyle (1987) with minor modification. About 0.3 g leaf tissue was ground to a fine powder in liquid nitrogen and mixed with 700 µL of CTAB (Cetyl Trimethyl Ammonium Bromide) extraction buffer (100 mM Tris-HCl (pH 8), 1.4 MNaCl, 20 mMEDTA (pH 8), 2% CTAB 1% β-mercaptoethanol, 1% PVP). The mixture was first incubated at 60°C for 90 min and then an equal volume of phenol: chloroform: isoamylalchol (25:24:1) was added, centrifuged at 12000 rpm for 20 min. The aqueous phase was decanted and transferred to a fresh microtube to reduce impurity between two phases. Extraction steps were repeated using phenol: chloroform: isoamylalcohol (25:24:1) mixture. The last aqueous phase was mixed with 2/3 of isopropanol and stored at -20°C for at least 2h to precipitate DNA and centrifuged at 12000 rpm for 15 min. The nucleic acid precipitate was washed with 70% ethanol, air-dried and suspended in 100 µL of ddH_2O. The extracted DNA was diluted in ddH_2O to 50 ng/µL and subjected to polymerase chain reaction (PCR).

DNA concentration was assayed with nano drop spectrophotometer (ND1000, USA) and quality was verified on 0.8 agarose gel electrophoresis.

RAPD-PCR amplification: RAPD-PCR was performed in a 25 µL volume using Gradiant Thermocycler (Eppendorf, Germany). Each reaction contained 25 ng template DNA, 10 pmol of primers, 300 mM dNTP, 25 mM $MgCl_2$, $(NH_4)_2SO_4$ buffer (1X) as PCR buffer and 0.5 unit Taq polymerase (Fermentase). Thermocycler conditions were 5 min at 94°C followed by 35 cycles of 94°C for 45 sec, 32°C for 30 sec and 72°C for 2 min.

SCAR-PCR amplification: PCR reaction for the SCAR marker were carried out with a final volume of 25 µL and 50 ng of genomic DNA, 10 pmol of each primer (PVF1 (Forward): 5′- GTCGTAGATGAAAACACC -3′, PVF2 (Reverse): 5′-TAATAGAAGCCATAGA -3′), 300 mM dNTPs, 25 mM $MgCl_2$, $(NH_4)_2SO_4$ buffer (1X) and 0.5 unit Taq polymerase (Fermentase). Amplification condition were: 1 cycle at 94°C for 2 min, 25 cycles at 94°C for 30 sec, 42°C for 15 sec, 72°C for 25 sec followed by 7 cycles increasing annealing temperatures in decrement of 0.4°C per cycle and finalized at 94°C for 30 sec, 45°C for 15 sec, 72°C for 15 sec and 72°C for 2 min to complete extension. Amplified products were then separated by electrophoresis in 1.2% agarose gel and stained with ethidium bromide. The expirement was replicated three times.

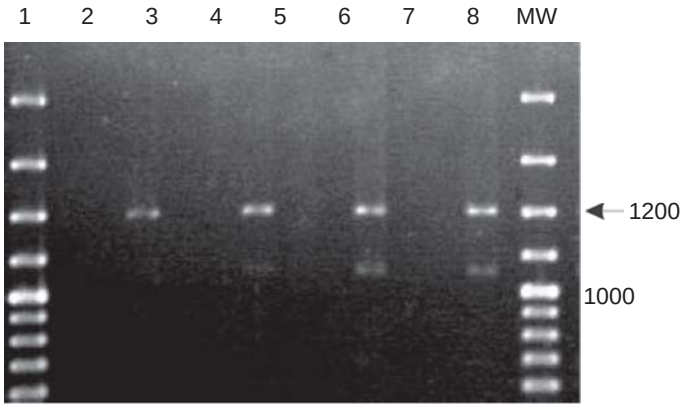

Fig. 1. A female-specific RAPD fragment in *P. vera* var Sarakhs amplified using primer OPO08. MW indicated size marker. The arrows denote the position of the 1200-bp fragment,

Results and discussion

After screening of 20 arbitrary 10-mer primers, we identified just BC1200 primer (5′GCCTGATTGC-3′) as being able to differentiate sex type (Fig. 1). This primer could amplify a weak 1200 bp fragment just in female *P. vera* var. Sarakhs. Amplification of genomic DNA from both male and female plants using SCAR primers showed that an approximately 300 bp fragment is present in all female samples of *Pistacia* species (Fig 2 and 3). By cloning and sequencing of RAPD marker (OPO08) and designing of the appropriate SCAR primers, a single specific band (297 bp segment) could be identified in female trees of *P. vera* (Yakubov *et al.,* 2004). Our study confirmed the results of Yakubov *et al.* (2004) in wild *Pistacia* species. In *Pistacia*, the genetic mechanism of sex identification is still unknown. The frequency of sex-linked markers depends on some factors such as the chromosome number, the total size of the genome and relative size of the chromosomal segments that determined sex. Mulcahy *et al.* (1992) reported four sex RAPD markers from screening of 64 random primers in *Silene latifolia*, which has a large Y chromosome; while Harvey *et al.* (1997) reported just two sex marker from testing of 500 RAPD primers. By screening of 1000 primers in *P. vera*, Hormaza *et al.* (1994) found just one sex marker. Kafkas *et al.* (2001) also found only two sex associated RAPD marker in screening of 472 primers. The low frequency of sex linked bands could indicate that loci gene which involved in sex determination is small and probably represents a single gene, or few genes. To learn about sex-inheritance and map distance measuring between a sex gene and the marker, segregating populations must be generated as test cross. Paran and Michelmore (1993) reported that sensitivity to reaction conditions and the requirement for high quality DNA, can hinder the use of RAPD markers, while SCAR primers amplify single band corresponding to genetically defined loci, are less sensitive to reaction conditions and have the capability of becoming co-dominant markers.

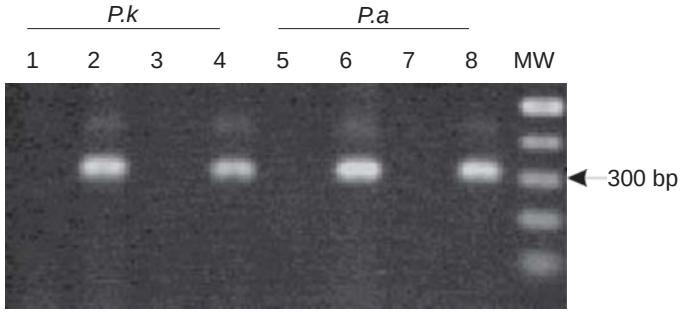

Fig. 2. SCAR pattern obtained from 8 genotypes of *P. khinjuk* (*P.k*) and *P. atlantica (P.a)* with PVF1 and PVF2 primers. Lanes 1-8 represent PCR-product of 8 trees. The arrow show the position of the 300 bp fragment which was present in female trees. The marker size (MW) is loaded before first genotype.

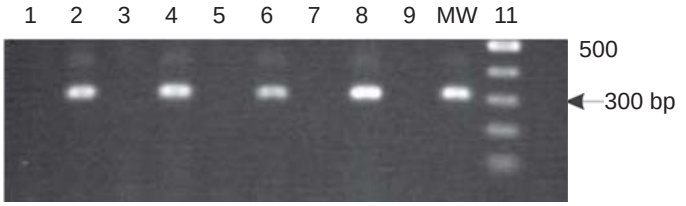

Fig. 3. Female - specific DNA fragments produced by PCR with PVF primers. Lanes 1-10 show band pattern of 10 genotypes of *P. vera* var. Sarakhs. Lane 11 show negative control.

Recent studies of sex determing mechanisms have illuminated clearly that angiosperm have evolved a variety of sex determining mechanism that involve a number of different genetic and epigenetic parameters, from sex chromosomes in *Marchantia polymorpha* and *Silene latifolia* to hormonal regulation in *Zea mays* (Peng *et al.,* 1999) and *Cucumis sativus* (Perl-treves, 1999).

Based on the association of molecular markers and sex, the presence of an XX/XY sex chromosome system was proved for *Hippophae rhamnoides* (Persson and Nybom, 1998), *Dioscorea tokoro* (Terauchi and Kahl, 1999), *Carica papaya* (Parasnis *et al.,* 1999) and *Actinidia* (Harvey *et al.,* 1997). By contrast, similar studies in *Atriplex garettii* suggest that sex determination involves a single locus on a homologous pair of chromosomes (Ruas *et al.,* 1998).

Despite increasing research efforts on a number of different plant species, there is relatively little information available on the molecular basis of sex determination. It is also difficult to estimate the number of genes involved in sex determination (Ainsworth, 2000).

Durand and Durand (1990) also reported that a single major gene controls sex determination in *Asparagus* and *Vitis*. *Pistacia* species might have a similar system with a major gene controlling sex determination. According to the results in *Pistacia* species, sex determination might be controlled by single locus acting as a trigger.

Quick sex determination would help farmers while selecting the female seedlings and maintain an optimum sex ratio at plantation, therefore, this will save time, economic cost and increase efficiency of pistachio breeding programs. Our study demonstrated that sex determination mechanism in pistachio might be controlled by single locus acting as a trigger. However, SCAR-PCR is a useful and reliable method to detemined sex genotype in *Pistacia* genus.

Acknowledgements

The authors thank H. Mirshahi for her assistance with various aspect of this research.

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Received: July, 2010; Revised: September, 2010; Accepted: November, 2010

Journal of Applied Horticulture, 12(2): 125-128, July-December, 2010

Genetic diversity of cultivated elephant foot yam (*Amorphophallus paeoniifolius*) in Kuningan, West Java as revealed by microsatellite markers

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Abstract

Ten microsatellite markers were used to clarify the genetic diversity of cultivated elephant foot yams collected in 13 villages in the Kuningan District, West Java, Indonesia. Each pair of primers generated four to five alleles, with an observed heterozygosity of 0.000-1.000 and an expected heterozygosity of 0.064-0.551. These markers identified seven likely genets (clonal individuals) in the Kuningan population. Of 61 individual plants surveyed in this study, 55 plants distributed throughout the Kuningan District belonged to the same genet, while the another genet represented by a plant (ramet). These ramets were restricted to the villages located on the main road between Kuningan City and Central Java. Cluster analysis shows that the seven genets can be classified into three groups, with two groups showing a restricted distribution in the villages located on the road leading to Central Java. Elephant foot yam plants with berries were rarely observed in the Kuningan District. It is likely that a single genet has become the dominated local cultivar, possibly because of the limited genetic diversity of elephant foot yam in the Kuningan District, its reproduction by clonal propagation and the selection of a specific cultivar by farmers.

Key words: *Amorphophallus paeoniifolius*, clonal propagation, cluster analysis, genet, genetic diversity, Indonesia, SSR

Introduction

Elephant foot yams [*Amorphophallus paeoniifolius* (Dennst.) Nicolson] are distributed in many Asian countries as a local tuber crop (Jansen *et al.*, 1996). Because elephant foot yam is a shade-loving plant (Santosa *et al.*, 2006), wild forms grow predominantly under medium and deep shady conditions in forests at altitudes up to 900 m above sea level (Jansen *et al.*, 1996). Elephant foot yams are cultivated in home gardens, upland fields and at the edge of paddy fields and bamboo forests, while semi-wild (escaped) plants are often found in riverbanks and teak forests.

Although distributed widely in Sumatra, Java, Madura, Bali, Lombok and Sulawesi, as well as other islands in Indonesia, corms of elephant foot yam are only consumed occasionally, for example as appetizers at lunch in rural areas in Java (Santosa *et al.*, 2002a; Santosa *et al.*, 2003). Corms are available during the dry season, when the plants are dormant. Few genetic studies have been carried out on elephant foot yams (Widjaja and Lester, 1987; Santosa *et al.*, 2002b). Sugiyama *et al.* (2006) found that accessions of Java elephant foot yams collected in the same subdistrict clustered together in a dendogram that depicted genetic distance based on AFLP polymorphisms. Elephant foot yams are usually propagated clonally using corms or cormels, and plants that clusterd very closely may all represent a single clonal lineage (genet) that has undergone somatic mutation. The authors considered that during the introduction of elephant foot yam cultivation, several clones with desirable agronomic traits and good taste were selected and cultivated on farms, while nonpreferred ones were abandoned. However, it was unclear how many clonal individuals (genets) existed within a region and how they were disseminated.

We evaluated genetic variation in cultivated forms of elephant foot yams in Kuningan District using microsatellite (simple sequence repeat, SSR) markers. Microsatellite markers are currently the most powerful markers for identifying the genets because they are characterized by hypervariability, high reproducibility and codominance (Ouborg *et al.*, 1999).

Materials and Methods

Field observations were carried out in Kuningan District, West Java, Indonesia, in December 2002 and July 2003, and villages where at least 20 farmers cultivated more than 10 large elephant foot yams (petiole diameter larger than 3 cm) each for over three years were selected. These villages were easily accessible by the main road, which runs from the mountainside (ca. 1500 m above sea level) to the foothills (ca. 200 m) of the southeastern slope of Mount Ciremai (3078 m) (Table 1, Fig. 1). The distance between villages was more than 2 km. In this area, elephant foot yams are cultivated in home gardens and at the edge of paddy fields, fishponds and upland fields. No wild population of elephant foot yams was found in the Kuningan District. Moreover, all growers, aged 48 - 66, stated that their parents or grandparents had already cultivated elephant foot yam in their villages. Leaflet sampling was carried out in December 2003.

Three to six plants with a petiole diameter larger than three cm were selected at random from each village at distance of at least 15 m, in order to reduce the chance of sampling the same genet

Table 1. Sampling sites of cultivated *Amorphophallus paeoniifolius* in Kuningan, West Java and the number of plants sampled at each site

Site number	Name of sampling site	Code	Number of plants sampled
1	Ciherang	CIH	5
2	Jambar	JAM	4
3	Haurkoneng	HAK	5
4	Tinggar	TIN	5
5	Bayuning	BAY	4
6	Kuningan	KUN	4
7	Cibinuang	CIB	5
8	Citangtu	CIT	6
9	Kedungarum	KAR	5
10	Mekarmukti	MMU	3
11	Mekarwangi	MWA	5
12	Luragunglandeuh	LLA	5
13	Luragungtonggoh	LTO	5
Total			61

Site numbers were the same as those used in the map of Fig. 1

(Table 1). One leaflet per plant (about 5 g) was removed from the tip of the tripartite leaf, and dried in plastic bags containing 50 g of silica gel (blue when dry). When the silica gel absorbed mositure and turned purplish pink, it was replaced with the dried silica gel. In total, the leaflets of 61 plants were collected from 13 villages. A cultivar sample from Yogyakarta was added as a reference.

DNA was extracted from dry leaflets with a modified cetyl trimethyl ammonium bromide method, and stored at –30 °C until used. Nineteen microsatellite loci (Ampa 1-19 developed by Santosa *et al.*, 2007) were screened. Eight loci (Ampa 1, 2, 8, 9, 10, 13, 14 and 18) were not amplified well and one locus (Ampa 16) produced a monomorphic band, so these were excluded from further analysis. The remaining 10 primers (Ampa 3, 4, 5, 6, 7, 11, 12, 15, 17 and 19) were used in the present study.

PCR was performed as described by Santosa *et al.* (2007). The PCR solution mixture (5 µL) contained 5-20 ng of template DNA, 0.5 µM of forward primer, 0.1 µM of reverse primer tailed with U-19, 0.5 µM of U-19 primer labeled with Texas Red, 0.2 mM of each dNTP mix, 1 × PCR buffer (Mg^{2+} free), 2.5 mM $MgCl_2$ and 0.5 U of Ampli *Taq* Gold DNA polymerase (Applied Biosystems, Foster City, USA). PCR using a PCR thermal cycler (Takara PCR system, Kyoto, Japan) was performed with the following cycling profile: 9 min at 94 °C, followed by one cycle of 30 s at the locus-specific annealing temperature plus 1 min at 72 °C, and then 38 cycles of 30 s at 94 °C, 30 s at the locus-specific annealing temperature plus 1 min at 72 °C, followed by one cycle of 30 s at 94 °C, 30 s at the locus-specific annealing temperature plus 5 min at 72 °C, and ending at 4 °C. The primer annealing temperatures are shown in Table 2. After denaturation by heating at 95 °C for 5 min, PCR products were immediately placed on ice and then electrophoresed on 6% polyacrylamide gel with 0.6 × TAE buffer, using a SQ-5500E sequencer (Hitachi Co., Tokyo, Japan). Electrophoretic patterns were analyzed with FRAGLYS ver. 3 software (Hitachi Electronics Engineering Co., Tokyo, Japan).

Genotypes that matched at all microsatellite loci were presumed to represent a single genet. For each identified genet, the observed (H_O) and expected (H_E) heterozygosity and the polymorphic information content (PIC) were calculated for each locus using CERVUS ver. 3 (Marshall *et al.*, 1998). The PIC provides information of the effectiveness in differentiating among genets; loci with higher values of PIC can distinguish genets more efficiently. A dendogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA) from a genetic similarity matrix using NTSYSpc (Rohlf, 2000). Bootstrapping with 1000 permutations was carried out using FreeTree software, according to Pavlicek *et al.* (1999). The inbreeding coefficient (F_{IS}), which describes the divergence of H_O from the H_E in panmixia populations, was calculated for each locus using FSTAT (Weir & Cockerham, 1984). Positive and negative F_{IS} values indicated significant excesses of homozygotes and heterozygotes, respectively.

Results and discussion

The number of alleles, heterozygosity and PIC of 10 microsatellite loci are given in Table 2. Either four or five alleles were observed (average allele number per locus was 4.4). H_O and H_E ranged from 0.000 to 1.000 and from 0.064 to 0.551, respectively. The PIC value ranged from 0.061 to 0.443 (0.262 on average). Some microsatellite markers had very low PIC, *e.g.*, Ampa 12 and Ampa 19 loci, suggesting that these markers could not discriminate genets in the Kuningan District efficiently. The average multilocus F_{IS} for all samples had a negative value (-0.702), showing an excess of heterozygotes.

Based on allelic data at 10 loci, 61 samples could be classified into seven genets in the Kuningan District. Of the seven genets, one genet (the main genet in Fig. 2) was represented by 55 sampled plants, while the other six genets were represented by one plant

Table 2. Locus name, simple sequence repeat (SSR) sequence, annealing temperature (T_a), number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (PIC), average inbreeding coefficient (F_{IS}) and gene diversity of microsatellite markers in cultivated *Amorphophallus paeoniifolius* from Kuningan, West Java

Locus	SSR Sequence	T_a (°C)	Number of alleles	H_O	H_E	PIC (%)	F_{IS}
Ampa 3	$(TG)_{16}$	56	4	0.984	0.504	37.5	-0.906
Ampa 4	$(CT)_7(GT)_{10}$	58	5	0.000	0.124	11.5	0.894
Ampa 5	$(TC)_{19}(TG)_{10}$	55	5	0.932	0.551	44.3	-0.657
Ampa 6	$(TG)_{18}(AG)_9$	60	5	1.000	0.528	41.0	-0.855
Ampa 7	$(TG)_{11}(AG)_{15}$	60	4	0.934	0.519	39.8	-0.761
Ampa 11	$(TC)_6(TG)_{14}$	60	4	0.951	0.511	38.6	-0.820
Ampa 12	$(TG)_{11}(AG)_{10}$	51	4	0.066	0.064	6.1	0.143
Ampa 15	$(GA)_7(GT)_{11}$	58	5	0.951	0.503	37.4	-0.847
Ampa 17	$(AG)_{12}(TG)_9(AG)_3$	57	4	0.951	0.503	37.4	-0.847
Ampa 19	$(GA)_7(GT)_8(CG)_6xx(CT)_{11}$	57	4	0.066	0.064	6.1	0.143
Overall			44				-0.702

each. Using allelic data for these seven genets, a cluster analysis was carried out using the UPGMA method. This divided the seven genets into three groups (Fig. 2). The main genet and the genets from Haurkoneng (HAK) and Luragunglandeuh (LLA) villages belonged to cluster I. The three genets from Luragungtonggoh (LTO) belonged to cluster II and the one genet from Mekarwangi (MWA) village belonged to cluster III. The four genets belonging to clusters II and III and one genet (HAK-4) belonging to different subgroup of cluster I from main genet were found only in the villages located on the road from Central Java to the centre of Kuningan City, whereas the main genet was distributed in all villages throughout the Kuningan District (Fig. 1). The elephant foot yam from Yogyakarta was genetically distant, joining with the Kuningan population at a similarity of 26%.

According to an interview with farmers in Kuningan, they usually remove inflorescences to produce big corms with good eating quality, when cultivating elephant foot yams. Removal of inflorescences of semi-wild elephant foot yams is a common practice in Kuningan as well as in other places in Java, because the inflorescences emit unpleasant odors. Plants with berries were only occasionally observed on the outskirts of Kuningan City, although evidence for berry set is common in the collection of elephant foot yams in the Herbarium Bogoriense.

In natural populations, the abundance of pollinators may affect the success of berry set. In Java, elephant foot yams can often produce inflorescences in mixed gardens where fruit trees, wood trees and medicinal plants were more extensively cultivated than in home gardens. Mixed gardens in Kuningan were usually separated by lowland paddy fields. Therefore, it is possible that only a few pollinators can carry pollen from one inflorescence to another inflorescence in elephant foot yam populations that are spatially isolated by paddy fields, leading to a scarcity of berry set in elephant foot yams in Kuningan. Although it is not clear whether the number of pollinators is large enough to ensure cross-pollination, a lack of outcrossing among plants may have restricted the expansion of genetic diversity of elephant foot yams in the present study area.

Geographically, LTO and MWA villages are located on the main road leading to Central Java (Fig. 1). There has long been the active movement of people, goods and information between Kuningan District and Central Java by this road. It is plausible that elephant foot yams have been introduced to these villages by merchants or other people from Central Java and Yogjakarta, where the cultivation of elephant foot yams has been more common (Kriswidarti, 1980; Santosa *et al.*, 2002a). A sugarcane plantation was established in LTO village in the 1800s, during the Dutch colonial period. This plantation was a powerful job creator and attracted a seasonal migration of laborers from Central Java. Sugarcane plantation laborers might have introduced several clones of elephant foot yam to Kuningan from Central Java or Yogyakarta. They could have brought either large corms of elephant foot yams as food, or small corms or cormels as planting materials. In the former case, laborers might discard or plant the skin of corms near their huts, after preparing corms for cooking.

A possible explanation for the dissemination of only one genet throughout the district and a limited distribution of other genets in villages close to the border of Central Java (*e.g.* LTO, MWA) is

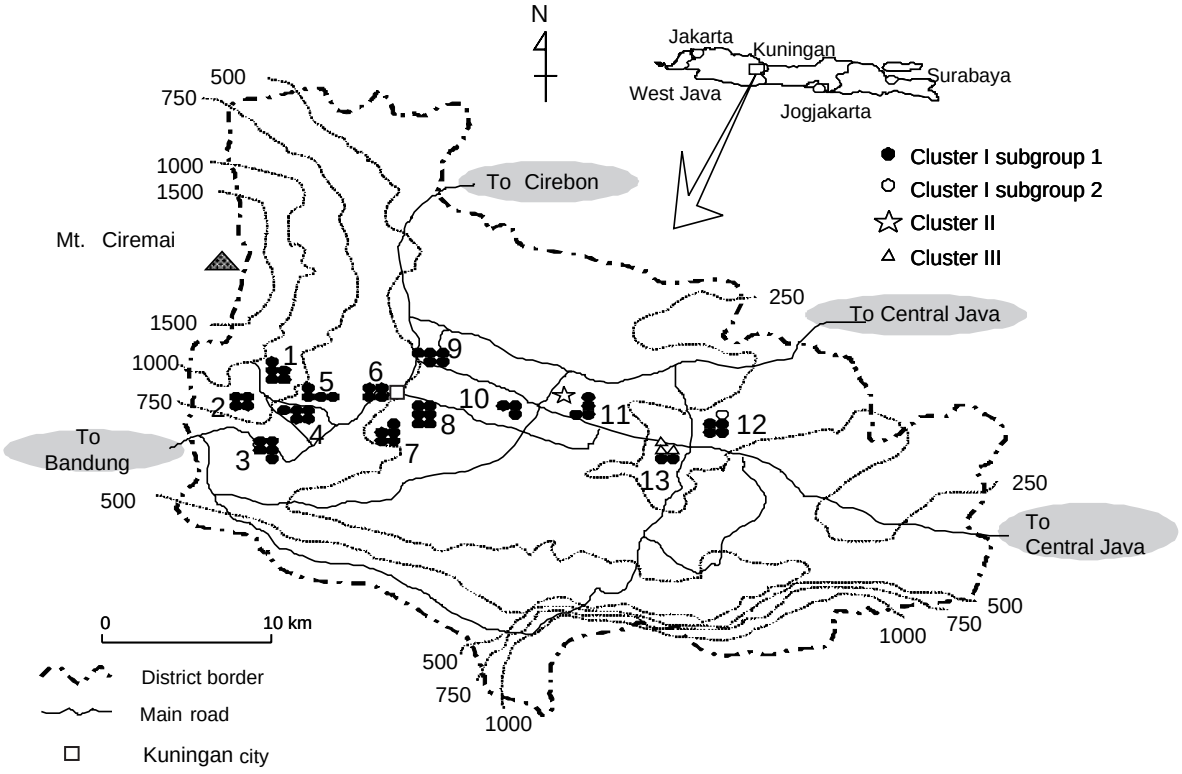

Fig.1. Distribution of elephant foot yam accessions in Kuningan District based on SSR data. Symbols represent cluster membership based on UPGMA method. Each circle or triangle represents a plant sample. Thirteen villages in Kuningan District, West Java, Indonesia for studying genetic variation of *Amorphophallus*. Main road referred to both provincial and district roads. Sampling sites were presented in Table 1.

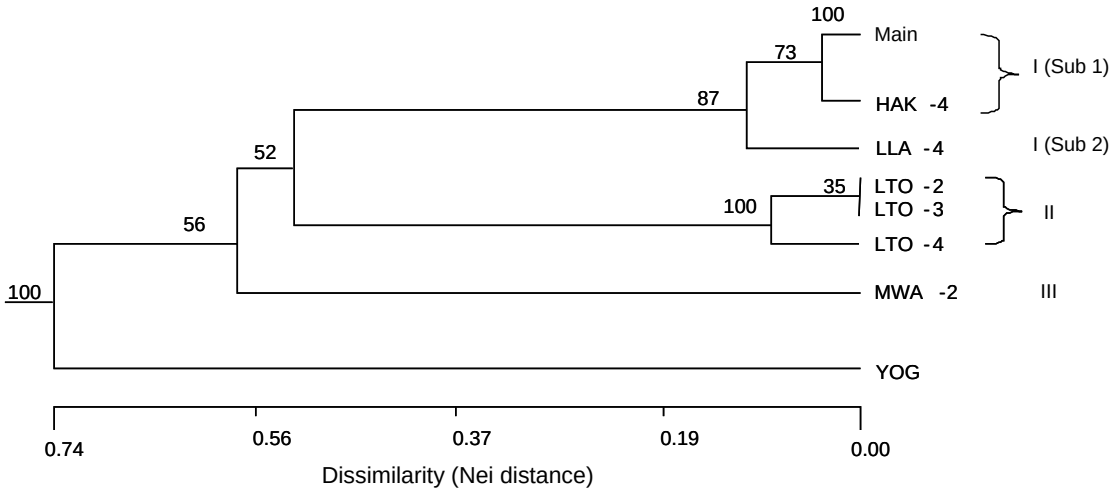

Fig. 2. UPGMA dendogram obtained from SSR markers data on 61 plants of *A. paeoniifolius* from Kuningan, West Java. Abbreviations are explained in Table 1. Main - samples other than HAK-4, LLA-4, LTO-2, LTO-3, LTO-4 and MWA-4; YOG - Yogjakarta. Bootstraping value from 1000 permutations. Roman figures are cluster numbers, and sub 1 and sub 2 in parenthesis mean the subgroup in cluster I.

that many clones (genets) were introduced to these villages from Central Java and given a evaluation by farmers based on their agronomic traits and taste. Possibly, most clones, *e.g.*, genets in clusters II and III, have been abandoned during selection, while only favoured clones might have been distributed throughout Kuningan in the form of cormels or corms. Santosa *et al.* (2003) reported that corms are usually exchanged among neighbours or relatives, and this is presumably how the corms of the cluster I genet gradually became distributed from person to person throughout the district. However, another possibility cannot be ruled out: clones (genets) belonging to clusters II and III might have been only introduced into Kuningan where the cluster I clone had already spread. Even if there is positive selection for the new clones, it must take time for farmers to adopt them. It is probable that Kuningan farmers are used to the taste of elephant foot yams which they have grown, and do not prefer new clones. However, Santosa *et al.* (2003) reported that merchants sold elephant foot yams transported from other districts at the Kuningan market. Therefore, it appears that Kuningan farmers do not necessarily prefer specific cultivars of elephant foot yams at the present time. Further study is necessary to investigatie the genetic variation in the semi-wild (escaped) plants throughout the Kuningan District, because many semi-wild clones belonging to clusters other than cluster I are expected to still remain in villages close to the border of Central Java if selection have been carried out in these villages.

Acknowledgements

This study was supported by a grant from the Japan Society for the Promotion of Science (JSPS) for Tropical Biological Resources.

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Received: May, 2010; Revised: September, 2010; Accepted: November, 2010

Journal of Applied Horticulture, 12(2): 129-134, July-December, 2010

Effect of grafting on vegetative growth and quantitative production of muskmelon (*Cucumis melo* L.)

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Abstract

Plants of muskmelon variety "Calypso" were used as scion and non grafted control while two hybrids (*Cucurbita maxima* x *Cucurbita mushata*), TZ148 and Ferro as rootstocks. Grafted and non-grafted plants were grown under a monotunnel heated and irrigated by geothermic water in the South of Tunisia. Plants were grown in soilless culture on sand and compost. This trial has revealed that, on sand as well as on compost, grafted plants were more vigorous than self-rooted ones. This vigor was highlighted by values of length and volume of roots, plant height, stem diameter, leaf area and fresh and dry matter of leaves. Indexes of growth represented by LAI, SLA, RGR and NAR were strongly improved by grafting particularly by TZ148. This improvement implied a hasty vegetative growth. Moreover, precocity of production was greater for grafted plants. In addition to their early production, grafted plants produced more fruits on sand and compost. The average weight of fruits was enhanced, too, by this agricultural practice. Thus, the major part of fruits produced by grafted plants had a weight superior to 600g.

Key words: Muskmelon, grafting, vegetative growth, indexes of growth, quantitative production

Introduction

In Tunisia, greenhouses cultivation is widespread specially because this system permits to produce out-off-season vegetables under controlled climatic conditions. In the northern part, these structures are unheated but in the South, they are developed ones, heated and irrigated by geothermic waters. Heating has permitted a gain of precocity and an amelioration of gustative quality that are limited under condition of low temperature (Mougou, 1987). Notwithstanding, it has created a favorable biotope for dissemination of pathogenic agents (Martyn, 1983) and amplification of salinity seeing the inner high evaporation. Indeed, few years after beginning farmers have complained these constraints.

Regarding to the agricultural, economical and social importance of this sector, Tunisia has aimed to overcome such hostile conditions of culture by adopting several practices like solarization, water washing, rotation of cultures and amendment of sand and organic matter. However, efficiency of these techniques was imperfect (Radhouani *et al.*, 2008).

In the world, many promising practices are adopted in order to surmount such constraints. Grafting is one of these techniques which is in root of becoming a popular agricultural practice. Khah *et al.* (2006) reported that Spain is the most important country for the spreading of vegetable grafting mainly with tomato and watermelon.

The use of grafted plants is considered an innovative technique which ameliorates vegetative growth (Jebari and Aounallah-Chouka, 1999; Zhusheng *et al.*, 2000; Rochdi *et al.*, 2005) and improves flowering (Lardizabal and Thompson, 1990). Consequently, productivity yield is increased (Wheaton *et al.,* 1995; Georgiou, 2000; Al-Jaleel *et al.*, 2005). Moreover, it was highlighted that this practice is able to conciliate plants with hard conditions of culture such as salinity (Edelstein *et al.*, 1999; Santa–Cruz *et al*., 2002; Fernandez *et al.*, 2004; Rochdi *et al.*, 2005; Ruiz *et al.*, 2006), low (Bulder *et al.,* 1990) and high (Rivero *et al.*, 2003; Estàn *et al.*, 2005) temperature, and drought (AVRDC, 2000). Besides, the use of grafted plants is seen as an alternative for chemical sterilization (Ginoux, 1993; Ginoux et Buffière, 1998) since it provides plants with resistance against soil-borne pathogens (Scheffer, 1957; Lee, 1994; Cohen *et al.*, 2005). Nevertheless, the reliability of grafting depends on interaction between rootstocks and scions as it was reported by Khah *et al.* (2006). Moreover, Romano and Paratore (2001) remarked that the choice of rootstock affects the effectiveness of this agricultural practice.

In this framework, the aim of this research was to evaluate the effect of two rootstocks on vegetative growth and production of muskmelon (*Cucumis melo* L.) cultured on soilless media under a greenhouse heated and irrigated by geothermic water in the South of Tunisia.

Materials and methods

Crop growth conditions: The experiment was conducted in the experimental field of the Institute of Arid Regions in Kebili (South of Tunisia). It was carried out in a mono tunnel (8.5 m of width x 30 m of length) covered by a white and 200 µm thick polyethylene film. Local sand and compost, formed by fermentation of dry palms with addition of manure, were used as substrates in this trial. Table 1 and 2 illustrated their main characteristics. The study was conducted in soilless media. Substrates were contained in plastic containers with a volume of 33 L. These containers were placed on ground settled down beforehand and covered by a plastic film. They were disposed on a fine layer of gravels. They were perforated to drain excess of water. Heating was realized by the circulation of geothermic

Table 1. Characteristics of local sand

Parameters	Value
Gravel (%)	2.7
Rough Sand (%)	46.3
Fine Sand (%)	48.3
Silt and Clay (%)	2.7
pH	8.2
EC (mS/cm)	2.5
Rate of retention of water (%)	26.0

Table 2. Characteristics of local compost

C/N (%)	Organic matter (%)	Total porosity (%)	pH	EC (mS /cm)	Rate of retention of water (%)
27.1	60	62.2	7.64	4.1	31

water (60°C) in corrugated polypropylene pipes (Ø 25) placed on the plastic between plant rows. The control of daily temperature was done by lateral aeration.

Plants were irrigated 4-5 times daily, depending on the size of the plant and the climatic conditions, by a drip irrigation system with one dripper per plant. To avoid salt accumulation in the substrate, plants were over watered once a week with geothermic waters without nutrients.

Plants material: Two commercial hybrids (*Cucurbita maxima x Cucurbita mushata*) TZ148 and Ferro were used as rootstocks with muskmelon, variety "Calypso" as scion.

A randomized complete block design was adopted with three replications: Two grafting combinations, Calypso grafted on TZ148, Calypso grafted on Ferro, and Calypso non-grafted was considered as control. Each replication was represented by eight plants on each substrate.

Measurements

Determination of vegetative growth: This growth was evaluated by measuring plant height, stem diameter, fresh and dry weight of leaves and leaf area.

Observations were recorded at 49, 64 and 79 days after transplantation. Measurents on leaves were recorded on the fifth leaf from the top. This choice was justified by the fact that it corresponds to a transformation from the state of well to source (Ouled Djeh *et al.*, 2006). Dry matter was obtained after drying the samples in an oven at a uniform temperature of 70ºC until a constant weight was obtained.

At the end of culture, plants were pulled out and length of the principal root was measured. Volume of roots was estimated as volume of water displaced by roots.

Calculation of growth indices: With dry weight data and leaf area, the following growth indices were calculated, according to Radford (1967) and Hunt (1978):

Leaf Area Index (LAI), which is the leaf area per surface area unit was calculated using the following formula:

LAI = Leaf area per plant x Number of plants/ m^2; expressed in cm^2/m^2

Specific Leaf Area (SLA), an indication of the thickness of leaf per unit of leaf area, was determined by the equation:

SLA = Leaf area per plant/ Leaf weight per plant; in $cm^2\ g^{-1}$ (dry weight)

Relative Rate of Growth (RGR), which reflects the ability of plant to produce a new dry matter in a specific period of time was calculated as following:

$RGR = d_W/W \times 1/dt$; in mg g^{-1} day^{-1}

W = dry weight of sample dt= d2 - d1 is the interval of time between samples of measure

Net assimilation rate (NAR), a measure of the biomass production by unit of leaf area during a specific period of time, was calculated as following:

NAR = (W2-W1) (LA2- LA1) x ln(LA2)-ln(LA1))/(d2-d1), ($g^{-1}m^{-2}day^{-1}$)

W2 and W1 = dry weight of sample; ln is the natural logaritm.

Evaluation of production: Days preceding harvest were counted in order to appraise precocity. The average number and weight of fruits were determined. Fruits were classed into three grades based on their weight (CTIFL, 1991): C_1: weight inferior to 600 g; C_2: weight ranging between 600 and 900 g; C_3: weight superior to 900 g. Total yield per substrate was determined.

Data analysis: An analysis of variance (ANOVA simple) was used to assess the significance of treatment means. Differences between the means of the three categories of plants were compared using the least significant difference (LSD) and Tukey test at the 0.05 probability level.

Results

Length of the principal root was not significantly affected by grafting yet the volume of roots was largely intensified by this technique (Table 3). This increment was around 55.58 and 38.1%, respectively on sand and compost. This effect was statistically similar for the two rootstocks. This behaviour of roots endowed by grafting was also highlighted by Rochdi *et al.* (2005). Rivero *et al.* (2003) have explained this effect by improvement of the meristematic activity.

Grafted plants were taller than self-rooted ones. Fig. 1 shows that the average height of plants was improved similarly by the two rootstocks on the two substrates. On sand, this improvement was not significant. Non-grafted plants had a height of 193 cm whereas grafted ones revealed values of 198.88 and 194 cm, respectively for TZ148 and Ferro. Conversely, on compost, grafting recorded an increment of 19% when compared to non-grafted plants which had reached a height of 185.22 cm. These results are similar with the findings of Georgiou (2000) and Khah *et al.* (2006), respectively for mandarin and tomato.

Furthermore, stem diameter increased as a result of this grafting

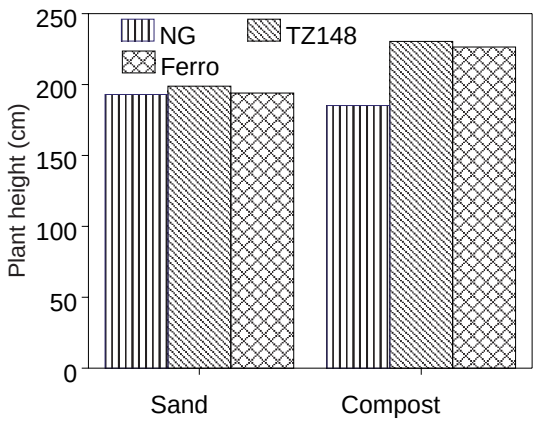

Fig. 1. Effect of grafting on plant height (cm)

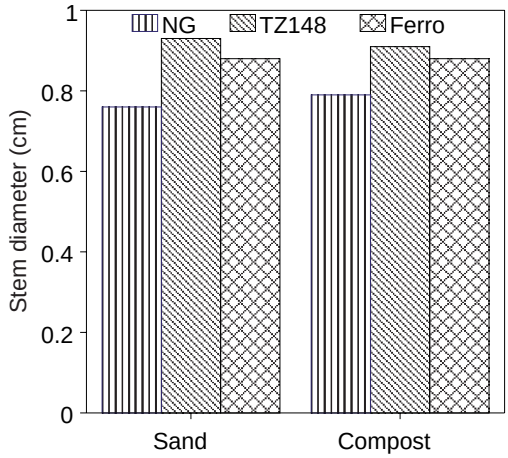

Fig. 2. Effect of grafting on diameter of the stem (cm).

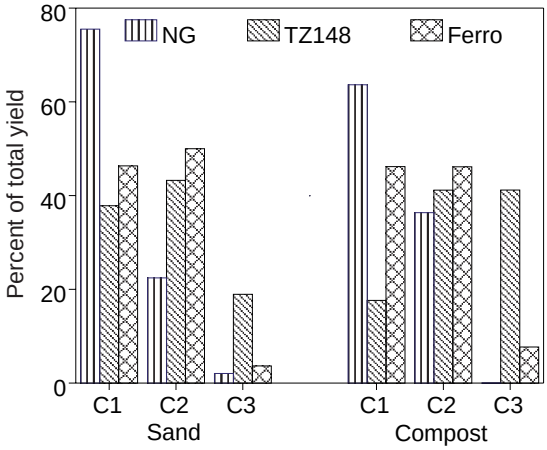

Fig. 3. Effect of grafting on grades of fruits.

technique (Fig. 2). For TZ148, this increment was around 18.27 and 13%, respectively on sand and compost. Ferro has enhanced this parameter by 13.63 and 10.22%, respectively on sand and compost. This observation confirms the findings of Lee (1994) and Ioannou *et al.* (2002) who have emphasized a tendency of grafted plants to attain a larger stem diameter.

It was observed that in both substrates grafting increased fresh weight of leaves. On sand, this effect was similar for the two rootstocks and was around 6.92%. On compost, this effect was more pronounced with TZ148 than Ferro. From the data presented in Table 4, it is seen that leaves of grafted plants, especially those grafted on TZ148, had a higher accumulation of dry matter than those of non-grafted ones. These finding corroborated with the reports on the effect of grafting on tomatoes by Khah *et al.* (2006).

Leaf area values, presented in Table 4, revealed that grafting induced production of larger leaves. However, this effect was not significant on sand. On compost, the increase was recorded for plants grafted on TZ148. Pulgar *et al.* (1998) attributed this

Table 3. Effect of grafting on growth of roots

	NG	TZ148	Ferro	Significance
	Average length of the principal root (cm)			
Sand	24.33a	36a	38a	NS
Compost	29.66a	41a	43.16a	NS
	Average volume of roots (mL)			
Sand	41b	93.33a	100a	***
Compost	59b	94a	96.66a	***

Values followed by the same letters within each line are not significantly different according to test of Tukey at $P < 0.05$.
Levels of significance are represented by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and NS, not significant.

amelioration to increased absorption, uptake and transmission of brute sap ingredients that were proved by Lee (1994), Oda (1995) and Al-Jaleel *et al.* (2005) in conditions of adoption of this practice.

Growth of leaves, represented by their fresh and dry weights and their mean area, was enhanced by grafting. This result was consistent with those indicated by Rochdi *et al.* (2005) for citrus fruits and Ruiz *et al.* (2006) for tobacco.

LAI, that constitutes a measure of leafiness per unit ground area of photosynthetic machinery (Amanullah *et al.*, 2007), was affected amply by grafting mainly on compost. Values illustrated in Table 5 showed that on sand the treatments had similar values of this parameter. While, on compost, until 64 days after transplantation, grafted plants, especially those grafted on TZ148, were leafier than non grafted one. Gaytán-Mascorro *et al.* (2008) have reported this remark for tomatoes and they noted that this situation demands pruning. Moreover, Pulgar *et al.* (1998) have remarked that grafting increased leaves production.

Seventy nine days after transplantation, non grafted plants showed a slight superiority against grafted one. This superiority may reflect a continued vegetative growth for this category of plants.

Referring to values of SLA (Table 5), it seems that on sand as well as on compost, until 64 days after transplantation leaves of grafted plants were thicker than those of self-rooted ones. This difference was greater with TZ148 than Ferro. On the contrary, 79 days after transplantation, leaves of grafted plants became thinner. This behavior may be due to the allocation of carbohydrates to prior organs, flowers and fruits.

The ability of plants to produce a supplement photosynthetic product, dry matter, in a specific period deducted from values of RGR was similar for three groups of plants cultivated on the two substrates (Table 5). However, values of NAR, which represents the efficiency of foliar area to produce new matter in a specific period, were fairly different between categories of plants. Thus, on two substrates non grafted plants showed a negative net assimilation whereas plants grafted on TZ148 exhibited positive

Table 4. Effect of grafting on growth of leaves

Parameter	Sand				Compost			
	NG	TZ148	Ferro	Significance	NG	TZ148	Ferro	Significance
Fresh weight (g)	1.68b	1.80a	1.81a	*	1.75b	2.16a	1.76b	**
Dry weight (g)	0.27b	0.29a	0.28b	*	0.28b	0.34a	0.26b	**
Leaf area (cm^2)	53.07a	55.1a	53.58a	NS	55.51b	65.98a	54.37b	**

Values followed by the same letters within each line for each substrate are not significantly different according to test of Tukey at $P < 0.05$.
Levels of significance are represented by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and NS, not significant.

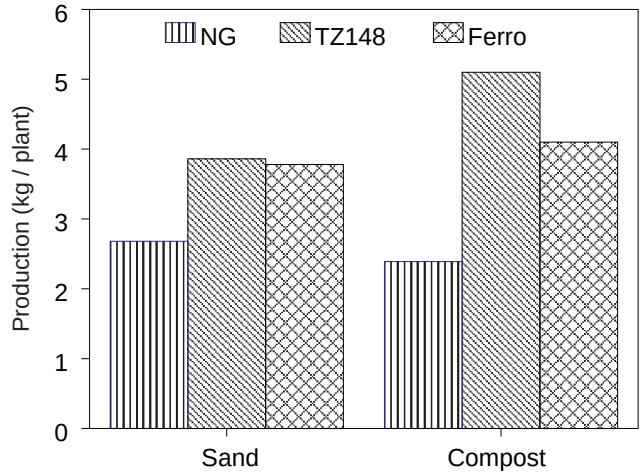

Fig. 4. Effect of grafting on total production (kg plant^{-1})

values of this parameter. The assimilation of plants grafted on Ferro was different on two substrates: on sand, it was positive while on compost, it was negative and more superior than those of non grafted plants. Negative values of this parameter may imply a minor assimilation that was not enough even for energetic expenses aroused by respiration. Indeed, Snelgar *et al.* (1980), Marcelis *et al.* (1998), Saadallah *et al.* (2001) and Loveys *et al.* (2002) have affirmed that net assimilation rate constitutes the final result of carbon's benefit by photosynthates and its release by respiration. This metabolic phenomenon is a major component of NAR. Consequently, it seems that the fifth leaf of non-grafted plants was a well (consumer of photoassimilates) not a source (producer of photoassimilates). This situation may reflect an extended vegetative growth hence a slight rate of this growth. Fahrurrozi (2000) has adopted this opinion, too, to explain negative values of this parameter for plants of muskmelon.

On sand, harvest was begun with plants grafted on TZ148 83 days after transplantation. Four days after, fruits of plants grafted on Ferro had been harvested and after a week, ripening of fruits of non-grafted plants was started. On compost, plants grafted on TZ148 were the first category of plants that produced fruits 86 days after transplantation. One and two days after this date became the ripening of fruits respectively for plants grafted on Ferro and non-grafted one.

Grafting increased the average number of fruits per plant (Table 6). This improvement was similar for two rootstocks but it was more prominent on compost than on sand. The maximum number of fruits per plant was 6.42 on compost against 5.66 on sand. Wheaton *et al.* (1995) indicated this effect for lemon trees and have assigned it to their more height.

The higher effect of grafting regarding to non grafted plants was observed, too, with the average weight of fruits that reached a maximum of 0.71 and 0.90kg with TZ148 on sand and compost, respectively (Table 6). Zhusheng *et al.* (2000) have also noticed this increase for orange.

This effect was inferred by more proportion of fruits with high weight (Fig. 3). Indeed, 62.17 and 82.36% of fruits produced by plants grafted on TZ148 respectively on sand and compost had a weight more than 600g (Class C2, C3). For plants grafted on Ferro, this rate was about 53.66 and 53.85%, respectively on sand and compost.

Table 5. Effect of grafting on growth indices

Substrate/ parameter		Date		
		49	64	79
LAI (cm^2/m^2)				
Sand	NG	6.18a	4.12a	2.63a
	TZ148	6.20a	4.44a	3.38a
	Ferro	6.05a	4.19a	3.23a
Compost	NG	4.97c	4.93b	3.97a
	TZ148	7.17a	6.14a	3.17a
	Ferro	5.85b	4.75 b	3.00a
SLA (cm^2/g)				
	NG	195.39a	254.19a	143.54c
Sand	TZ148	177.23b	193.46c	202.85a
	Ferro	181.52b	215b	185.0b
	NG	219.62a	246.75a	158.9c
Compost	TZ148	180.10c	210.74b	190.65a
	Ferro	198.83b	218.73b	180.2b
RGR (mg/g/day)				
	NG	45.50a	32.47a	26.10a
Sand	TZ148	44.52a	32.31a	26.70a
	Ferro	45.11a	31.17a	26.14a
	NG	42.42a	32.11a	23.56a
Compost	TZ148	40.00a	30.00a	25.00a
	Ferro	-	-	-
NAR ($mg/cm^2/day$)				
	NG	-6.90c	-3.60c	-3.10b
Sand	TZ148	1.77b	1.34b	1.01a
	Ferro	5.80a	4.30a	3.40a
	NG	-4.13b	-3.14b	-2.53b
Compost	TZ148	7.60a	5.70a	4.60a
	Ferro	-2.60b	-1.60a	-1.20a

Values followed by the same letters within each line for each substrate are not significantly different according to test of Tukey at $P < 0.05$.

Table 6. Effect of grafting on production

Media	NG	TZ148	Ferro	Significance
	Average number of fruits (fruits/plant)			
Sand	5.32a	5.44a	5.66a	NS
Compost	5.28b	5.71ab	6.42a	*
	Average weight of fruits (kg/plant)			
Sand	0.5b	0.71a	0.66a	*
Compost	0.45b	0.90a	0.64ab	**

Values followed by the same letters within each line are not significantly different according to test of Tukey at $P < 0.05$.

Levels of significance are represented by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and NS, not significant.

Consequent to these improvements, total production was clearly benefited by grafting (Fig. 4). On two substrates, this increment was more prominent for TZ148. Thus, on sand, plants grafted on this rootstock exhibited a superiority of 30.56 and 2.07%, respectively in relation to non grafted ones and those grafted on Ferro. On compost, this superiority was around 53.13 and 14%, respectively. This enhancement confirms the previous findings for muskmelon (Edelstein *et al*., 1999; Jebari and Aounallah-Chouka, 1999; Cohen, 2006), tomatoes (Estàn *et al.*, 2005), lemon (Wheaton *et al.,* 1995; Al-Jaleel *et al.*, 2005) and mandarin (Currie *et al.*, 2000; Georgiou, 2000).

This study showed that grafting of muskmelon has positive effects on the performance by improving vegetative growth due to vigorous roots that favoured considerable uptake of water

and nutrients and rate of growth deduced, especially, from high values of RGR and NAR. Consequently, production was earlier and higher. These effects are dependent on choice of suitable rootstocks and condition of crop growth.

In Tunisia, where mostly cultivation under heated greenhouses is still conducted in soils, grafting seems to be a useful practice especially when media are saline and infested by many pathogens. Therefore, soilless culture shows a promise for production in greenhouses.

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Received: December, 2009; Revised: June, 2010; Accepted: October, 2010

Journal of Applied Horticulture, 12(2): 135-139, July-December, 2010

Quality and physiological responses of Fuji apple to modified atmosphere packaging during cold storage

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Abstract

Modified atmosphere packaging (MAP) with polyolefin bags made of modified polyvinyl chloride (mPVC), micro-perforated polyethylene (mpPE), modified polyethylene (mPE), plastic film mulch (control-1), and polyvinyl chloride with holes (control-2) were evaluated for their ability to preserve quality of Fuji apple during storage at 0 to 1°C. The results showed that atmosphere in mPVC bag was adjusted to 2.73%~2.38% CO_2 and 15.70%~18.13% O_2 while in mpPE, mPE and control-1 bag CO_2 levels were elevated and O_2 level declined to 0.10~0.72%，20.53~20.9%, respectively. In mPE bag, fruits recorded significantly less weight loss than other packagings throughout the storage, while fruit in mPVC, fresh weight loss was same as in control-1. The overall fruit quality of flesh firmness (FFF), soluble solid content (SSC) and ascorbic acid remained at almost the same level in each packaging during the first 40 days of storage, and changed thereafter. Control-1 resulted in significantly lower FFF than other packagings till day 220 and SSC showed the same trend as in control-2. Respiration rate of fruit in mPVC, control-1 and control-2 peaked on day 220 and those in mpPE and mPE peaked on day 240. Ethylene production of fruit in each packaging increased since day 40 and peaked on day 80 for mPE and control-1, day 100 for mpPE and control-2, on day 120 for mPVC. A second peak for mPE appeared on day 120. Each packaging resulted a dramatic increase and drop of SOD activity in fruit in the first 40 days. After about 220 days of storage, superficial scald and core browning occurred on fruit in mpPE, mPE, control-1, control-2 by 2.4~6.0% and 1.2~1.6%, 6.3~7.9% and 15.8~17.3%, 0~1.6% and 4.4~4.6%, 15.4~16.1% and 3.2~4.5%, respectively while no such incidence was observed in mPVC. Decay and disorder developed faster when storage duration increased.

Key words: Polyolefin film, scald, core browning, respiration rate, ethylene production, modified atmosphere packaging.

Introduction

Fuji is the most important, late-maturing apple cultivar in China. Covering fruit with paper bags during fruit development ("bagging") is now a common practice in response to market demands for reducing pesticide use. However, bagging may result in Fuji apples with thinner skin, less wax, and wax platelets that are arranged looser than in fruit not bagged (Li *et al.*, 2008; Liang and Huang, 2009). This results in poor quality retention and storage life of the fruit after harvest.

The use of modified atmosphere packaging (MAP) made with polyolefin films and holding at low temperature has been used successfully as a lower-cost alternative to controlled atmosphere (CA) storage for reducing decay and extending storage life of many fruits. However, greater caution is required when using MAP on Fuji apples because they are susceptible to CO_2 injury (Tian *et al.*, 2008). Previous research established that atmospheres containing 1.8-5.0% CO_2 and 12-19% O_2 at 0±0.5 °C are generally optimum for long term storage of Fuji apples (Guan *et al.*, 2004; Yang *et al.*, 2004). Xu *et al.* (2006) reported that ultra low-oxygen (1.6% ± 0.1% O_2) and 0.2% CO_2 reduced the incidence of superficial scald while maintaining acceptable quality after 100-day storage at 0℃. However，few studies have evaluated different polyolefin bags used to package apple fruit on their ability to maintain apple quality during storage, causing confusion in the market about which bag works best for Fuji apples. New types of breathable polyolefin bags made of modified polyvinyl chloride (PVC) or polyethylene (PE) were evaluated and compared with polyolefin bags currently used commercially for long-term storage of Fuji apples.

Materials and methods

Fruit and their handling: Uniform Fuji apple fruit of Chang-Fu No.2 variety with diameter of about 75 mm were harvested from 15-year old, healthy trees on 18 October, 2007 and 14 October, 2008 at the Baishui base of Apple Research Centre, Shaanxi, China. The trees were grown using normal commercial cultural practices. Developing fruit were covered with two-layer-paper KOBAYASHI bags [KOBAYASHI (Qingdao) CO., LTD, China] in mid-June in both years and bags were removed 6 days before harvest. Harvested fruit were wrapped with a PVC foam net (Jingfeng foam net factory, Xianyang, China), loaded in Waleng Paper Boxes (480×320×320mm, Xi'an shengda packing CO., LTD, China) and transported about 150 km by truck to the laboratory and immediately pre-cooled for 24 h using room-cooling at 0 °C.

Packagings and storage: After pre-cooling, the net wraps were removed and fruit of uniform size, colour and free of visible defect

or disease were selected for further treatments. About 5 kg of fruit (23-25 apples) were packed into a 65×65 cm bag made with one of the following five different polyolefin films: 1) modified polyvinyl chloride (mPVC), 2) micro-perforated polyethylene (mpPE), 3) modified polyethylene (mPE), 4) linear low density polyethylene plastic film mulch (PFM, commercial MA control, named control-1), or 5) polyvinyl chloride with eight, 10 mm diameter holes per bag (PVC; commercial non-MA control, named control-2). Each packaging was tied with plastic bandage to make the mouth of each bag air tight, leaving almost no void space between the top and the fruit. The permeable properties for each non-holed polyolefin bag are shown in Table 1.

Table 1. Parameters of polyolefin films used in the current study**

Type of bag*	mPVC	mpPE	mPE	Control-1
Thickness (mm)	0.035	0.020	0.010	0.006
O_2 transmission rate (mL/m^2 d·atm)	6.80×10^3	2.57×10^5	1.50×10^4	1.54×10^4
CO_2 transmission rate (mL/m^2 d·atm)	4.32×10^3	2.48×10^5	7.30×10^4	6.53×10^4
Transmission ratio of CO_2/O_2	6.350	0.967	4.9	4.23
water vapour transmission rate (g/m^2 d)	25	13.4	4.48	22.8

*mPVC: modified polychloride film; mpPE: micro-perforated polyethylene film; mPE: modified polyethylene (mPE); Control-1: plastic film mulch.

**Data were provided by National Engineering Technology Research centre for Preservation of Agricultural Products, China. All parameters were measured under RH=50%, 20℃.

During the 2007-2008 seasons, each treatment consisted of 10 bags and weight loss and decay were investigated at the beginning and end of storage. In 2008-2009, each treatment consisted of 19 bags; thirteen for evaluations every 20-day, six for the investigation of weight loss and decay. All packaged fruit were held in ventilated plastic boxes (60×40×40cm, 2 bags per box) and stored at 0 to 1°C.

Measurements of FFF, SSC, TA and ascorbic acid: At each sampling time in 2008-2009, 10 fruit were removed randomly from 3 polyolefin bag per treatment to measure fruit flesh firmness (FFF), soluble solids content (SSC), titratable acidity (TA) and reduced ascorbic acid (AA). For FFF, about a 15 mm diameter area of peel was removed from three equidistant locations around the equator of each fruit and the maximum penetration force was measured in Newtons (N) using a firmness tester (GY-3, Zhejiang, China) with an 11 mm cylindrical probe moving 5 mm s^{-1} and penetrating 10 mm into the flesh. A longitudinal slice of flesh was then removed from each peeled spot and squeezed to produce juice that was measured for SSC using a fruit sugar tester (WY032T, Sichuan, China). The three FFF and SSC measurements were each averaged per fruit and recorded as the value of one fruit. Longitudinal apple slices of more than 30 g fresh weight that excluded both core and peel were cut from the remaining areas of each fruit, combined with slices from the 10 fruit for each treatment, separated into three replicates of 30 g each for TA analysis and 50 g each for AA analysis, and then froze immediately with liquid nitrogen. Remaining apple flesh of the three replicate were also frozen and held at −80 °C until later analysis of SOD and MDA.

TA was determined using the method described by Akbudak and Eris (2004) with some modification as: a total 100 mL suspending sample solution was heated to 80℃ in a water bath for 30 min, centrifuged at 1500 ×g for 15 min, and then filtered through Whatman 541 filter paper from which 20 mL of the supernatant was titrated with 0.1M NaOH to an endpoint of pH 8.2 (pH-meter PHS-3c, Mettler Toledo, Germany). Results were expressed as g of malic acid per 100 mL. In the same way, additional supernatant was prepared from 50 g of fresh material to measure AA content according to the official titrimetric method (AOAC, 1990).

Respiration rate and ethylene measurement: At each sampling time in 2008−2009, an additional three replicates of 3 fruit each per treatment were randomly selected from the same 3 bags as previously chosen. The fruit were weighed; each replicate placed into its own 2.5 L jar, and respiration and ethylene production measurements were taken at 0 to 1°C. Respiration rate (RR) was determined using a flow-through system with a fruit-vegetable respiration analyzer (GXH-3051H, Jun-Fang Hi-tech, China) equipped with an Infra-red CO_2 detector.

Immediately after RR determination, replicates of fruit were then sealed in 2.5 L jars for 1 hour, and headspace was evaluated for ethylene accumulation using a gas chromatograph (GC-14A, Shimadzu Corporation, Japan) equipped with a flame ionization detector (FID) and 90 °C oven temperature, 130 °C injector temperature, and 250 °C detector temperature. An empty container was used as a blank. After each measurement the volume of the three fruit was determined using water displacement.

Measurement of SOD activity and MDA content: About 1g of frozen sample was used to determine superoxide dismutase (SOD) activity and 5 g for malondialdehyde (MDA) content, both following the method of Wang *et al.* (2005)

Measurement of O_2 and CO_2 concentration in bags: Headspace of polyolefin bags made of the different treatment materials and filled with fruit was evaluated for O_2 and CO_2 concentration using an O_2 and CO_2 analyzer (accuracy: 0.01%, Checkmate 9900, PBI Denmark) on day 20 and day 220 of storage. Sample gas was taken by a syringe through a pre-pasted rubber pad.

Fruit weight loss rate and decay measurements: Weight loss was evaluated by comparing fruit weight at each sampling date to their initial weight before storage. Fruit decay and physiological disorders were evaluated after 220 days of storage during the 2007−2008 season, and after 220 and 270 days of storage during the 2008−2009 season. Results were expressed on a percentage basis.

Statistical analysis: All statistical analyses were performed with SPSS 17.0. ANOVA was used to compare difference between treatments (including control), and Duncan's test was applied to compare differences between means when ANOVA showed significant differences. Differences at $P < 0.05$ were considered to be significant.

Results

Effect of polyolefin bags on internal O_2 and CO_2 concentration: O_2 and CO_2 levels within the two control bags were similar to atmospheric conditions and did not change between 20 and 220 days of storage at 0 to 1 °C (Table 2). In contrast, three of the sealed polyolefin packagings developed significantly increased internal CO_2 levels and occasionally significantly decreased O_2 levels. In particular, mPVC bags resulted in a more extensive

Table 2. Internal O_2 and CO_2 concentrations during storage within polyolefin film bags containing approximately 5 kg of Fuji apples

	Day	Type of bag *				
		mPVC	mpPE	mPE	Control-1	Control-2
CO_2(%)	20d	2.73±0.015 a	0.17±0.06c	0.40±0.06b	0.10±0c	0.03±0c
	270d	2.38±0.016 a	0.31±0b	0.72±0.058b	0.10±0c	0.02±0c
O_2(%)	20d	15.70±0.7 a	20.70±0.05b	20.53±0.35b	20.57±0.37b	20.93±0b
	270d	18.13±0.67 a	20.90±0.05b	20.32±0.4b	20.80±0.53b	20.93±0b

*mPVC: modified polychloride film; mpPE: micro-perforated polyethylene film; mPE: modified polyethylene; Control-1: plastic film mulch; Control-2: polyvinyl chloride with eight 10 mm diameter holes per bag.
**Different small letters followed each value represent significant difference between treatments according to the LSD multiple range test ($p < 0.05$).

Table 3. Weight loss (%) of Fuji apples in polyolefin film bags after different storage durations

Type of bag *	Storage duration (days)								
	0	20	40	60	80	100	160	200	220
mPE	0	0.39a**	0.03a	0.04a	0.08a	1.5a	2.05a	3.79a	5.08a
mpPE	0	0.32a	0.59a	0.83ab	1.22ab	4.4b	5.4b	7.58b	7.82b
Control-1	0	1.15a	1.64a	2.01b	2.53b	4.6b	6.09b	7.59b	8.05bc
mPVC	0	2.38b	3.49b	3.97c	4.23c	5.4b	6.54b	7.83b	8.25bc
Control-2	0	0.29a	3.91b	4.96c	4.61c	5.54b	6.47b	7.17b	9.62c

*mPVC: modified polychloride film; mpPE: micro-perforated polyethylene film; mPE: modified polyethylene; Control-1: plastic film mulch; control-2: polyvinyl chloride with eight 10 mm diameter holes per bag.
**Different small letters followed each value represent significant difference between treatments according to the LSD multiple range test ($p < 0.05$).

modified atmospheres (MA) compared to other MAPs after both 20 and 220 days. Between day 20 and day 220, O_2 increased 2.43% and CO_2 decreased by 0.35% in mPVC bags. This agrees with Guan *et al.* (2004) who reported O_2 increased 2.0% and CO_2 decreased slightly in mPVC packagings between 10 to 100 days at 0 °C. Use of mPE resulted in CO_2 levels doubling and O_2 levels decreasing by 0.17 to 0.58% compared to mpPE. However, both mPE and mpPE have greater gas permeability (Table 1) and, thus, less of a MA developed inside the bags than when mPVC was used. The gas permeability of control-1 was the greatest of the bags tested and resulted in no significant difference in CO_2 or O_2 compared to control-2. To extent, no real MA-effect was developed by control-1(PFM bag).

Fruit quality responses of Fuji apple stored in the different polyolefin bags: Table 3 shows that apples in mPE bags lost significantly less fresh weight than those in other packagings after 100-day storage. This trend remained same until day 220 when weight loss from control-2 became significantly more than other packagings including control-1. Each sealed packaging retained > 92.8% of the original apple fresh weight after 220 days of storage, which was 1.4 % higher than the hole-opened control-2. No visible shrinkage was noticeable on apples from any of the sealed treatments.

Fruit flesh firmness (FFF) within all packagings declined gradually with storage duration and there were few significant differences between treatments at each sampling period (Table 4). Only on day 220, mpPE and control-2 were significantly firmer than mPVC, mPE and control-1.

In the first 180 days of storage, all SSC values declined slowly, then it began to decline more quickly. There were no significant differences in fruit SSC among the different treatments at each sampling period until the last evaluation on day 220 when mpPE had the highest SSC, and control-2 had the lowest (Table 4).

Titratable acidity (TA) levels steadily declined during storage from an initial value of 0.37 to 0.09 g $100g^{-1}$ after 220 days (data not shown), but there were no significant differences among treatments. Overall, there were few differences in apple ascorbic acid (Vc) content within each packaging. Only after 100-day storage Vc content of apples in mPE, control-1 and control-2 decreased faster than the other treatments, but after 140 days these differences disappeared.

Postharvest physiological responses of Fuji apple stored in the different polyolefin bags: Apple respiration rate (RR) was variable until 200-day storage when climacteric peaks began to appear (Fig.1). Fruit within control-1, mPVC and control-2 bags peaked on about day 220, whereas those in mPE and mpPE appeared to peak on day 240.

Peak ethylene production in apples held in control-1 and mPE bags occurred earliest after 80-day storage, followed by apples in mpPE, mPVC and control-2 bags after 100 days (Fig. 2), which corresponded with the accelerated loss of fresh weight and firmness. The peak in ethylene production was longest in apples held in mPE bags and lasted from 80 to 120 days during storage.

Superoxide dismutase (SOD) activity peaked dramatically from 125 to about 540 iu g^{-1} h^{-1} after 20-day storage before dropping

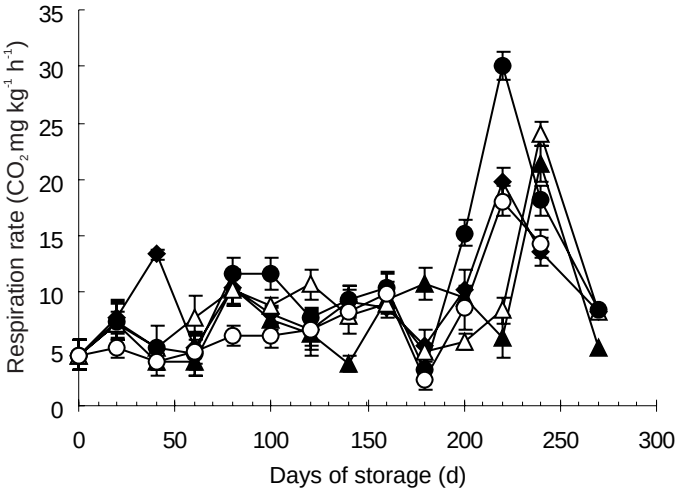

Fig. 1. Respiration rate changes of fruit from each packaging with storage time. Leagend: mPVC(♦), mpPE (▲), mPE(□), Control-1 (●) and Control-2(○)

Table 4. Changes in Fuji apple flesh firmness, soluble solids and ascorbic acid content during storage in different polyolefin bags

Storage duration (days)	Type of bag*	Firmness (N)	Soluble solid content (%)	Ascorbic acid content (mg 100g^{-1} FW)
0		78.05a**	15.49a	5.20a
40	mPVC	76.7ab	15.35ab	5.24a
	mpPE	74.7ab	15.36ab	4.95a
	mPE	70.7ab	15.52ab	5.26a
	Control-1	73.3ab	15.23ab	4.96a
	Control-2	70.4b	15.14ab	5.02a
100	mPVC	65.9bc	15.03b	4.20b
	mpPE	72.0ab	15.28ab	4.30b
	mPE	69.7b	15.18ab	2.65cd
	Control-1	68.3bc	14.64bc	3.20c
	Control-2	68.7b	14.86bc	3.30c
140	mPVC	66.0bc	15.17ab	2.90cd
	mpPE	69.7b	13.98d	3.77bc
	mPE	67.0bc	15.21ab	3.11cd
	Control-1	68.0bc	14.12cd	3.33c
	Control-2	67.0bc	14.59bc	2.90cd
180	mPVC	65.0bc	14.86bc	2.33d
	mpPE	67.3bc	14.75bc	2.93cd
	mPE	65.6bc	14.28cd	2.88cd
	Control-1	61.3cd	14.67bc	2.66cd
	Control-2	66.8bc	14.50c	2.77cd
220	mPVC	60.8cd	13.20e	2.23d
	mpPE	64.4bc	13.78d	2.54cd
	mPE	61.1cd	13.02e	2.24d
	Control-1	56.3d	13.00e	2.07d
	Control-2	62.4c	12.16f	2.16d

*mPVC: modified polychloride film; mpPE: micro-perforated polyethylene film; mPE: modified polyethylene; Control-1: plastic film mulch; control-2: polyvinyl chloride with eight 10 mm diameter holes per bag.

**Different small letters followed by each value represent significant difference between treatments according to the LSD multiple range test ($P < 0.05$).

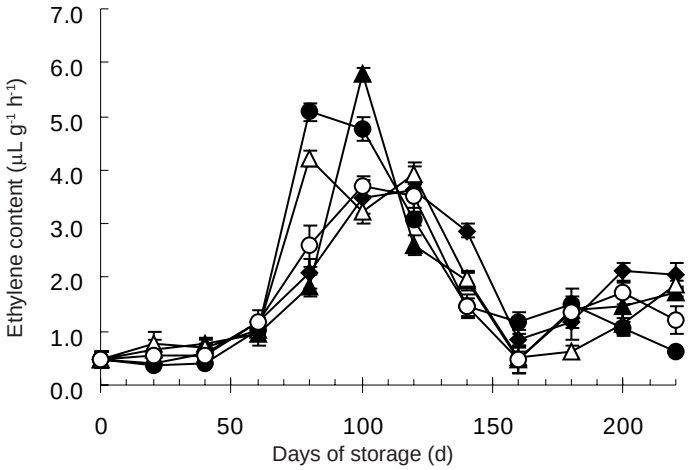

Fig. 2. Ethylene production changes of fruit from each packaging with storage time [mPVC (♦), mpPE (▲), mPE (□), Control-1 (●) and Control-2 (○)]

back to about 76 iu g^{-1} h^{-1} on day 40 (data not shown). Levels fluctuated at low levels thereafter. There were no significant differences between packagings.

Malondialdehyde (MDA) content of apples increased from 1.0 mmol g^{-1} FW^{-1} to 8.3 mmol g^{-1} FW^{-1} during the first 120 days of storage (data not shown). There were two main periods of rapid increase during this time spanning between 20−40 d and 80−120 d. After this, MDA levels decreased slightly.

Decay and disorders of Fuji apples stored in the different polyolefin bags: Over two seasons after 220 days of storage, the incidence of decay and core browning was consistently most severe on fruit held in mPE bags, whereas superficial scald developed most severely on apples held in mpPE, mPE, and control-2 bags (Table 5). As storage duration increased to 270 days during the 2008−2009 season, decay and disorders increased, but fruit in mPVC bags usually developed significantly less scald and core browning than other treatments as well as controls.

Discussion

Reports on peaches (Akbudak and Eris, 2004) and Teng-Mu No.1 apple (Liu and Ren, 2009) revealed that the maintenance of FFF within polyolefin bags is related to reduced water loss. In the current investigation, apples held in mPVC and control-1 bags lost significantly more fresh weight than other MAPs during 220

Table 5. Development of decay, scalds and core browning of Fuji apples after storage in different polyolefin film bags

Disorder	Storage duration (days)	Season	mPVC*	mpPE	mPE	Control-1	Control-2
Decay fruit rate (%)***	220	2007-2008	0.0a**	0.0a	6.3b	0.0a	0.0a
		2008-2009	0.0a	0.0a	4.2b	0.0a	0.0a
	270	2008-2009	3.2a	1.2a	23.9b	4.7a	7.5a
Scald fruit rate (%)****	220	2007-2008	0.0a	6.0b	7.9b	1.6ab	15.4c
		2008-2009	0.0a	2.4ab	6.3b	0.0a	16.1c
	270	2008-2009	30.9a	59.5b	71.2c	40.1a	50.6b
Core browning rate (%)	220	2007-2008	0.0a	1.6a	15.8b	4.6a	4.5a
		2008-2009	0.0a	1.2a	17.3b	4.4a	3.2a
	270	2008-2009	25.0a	35.9b	40.3b	39.4b	34.8b

*mPVC: modified polychloride film; mpPE: micro-perforated polyethylene film; mPE: modified polyethylene; Control-1: plastic film mulch; control-2: polyvinyl chloride with eight 10 mm ø holes per bag.

**Different small letters followed each value represent significant difference between treatments according to the LSD multiple range test ($p < 0.05$).

Fruit with visible deteriotion excluding scald defect stands for decay fruit which was counted immediately after each bag was removed from store room. * Scald fruit was counted 24 h after each bag was removed from store room.

days of storage, which was associated with the higher moisture permeability of the two bag material. However, in mPE bag, significantly less fresh weight loss was recorded than all other bags throughout the storage; it developed the same low FFF on apples as mPVC and control-1 in the end of storage; mpPE resembles mPE in gaseous permeability, but higher moisture permeability resulted significant higher apple FFF than mPE. This suggests that too much moisture in Fuji apple may obscure the MA-effect from packaging and enhance FFF drop as the same as too much water loss (control-1). Meanwhile, on day 40, FFF, SSC and AA were all at par as on 0 day for each packaging except for control-2, and then declined as storage time increased (Table 4). It revealed that Fuji apple was able to maintain a primary quality for at least 40 days with respect to physical and chemical changes under sealed packages and conditions of this work.

The initiation of the apple respiratory climacteric corresponded to a reduction in SSC in most packagings (Table 4). Apple RR peaks on day 220 coincided with significant decreases in SSC of fruit in control-1, mPVC and control-2 bags. These results support the fact that respiration consumption causes the decline of SSC in Fuji apple. Fruit in mPE bag didn't follow this rule due to the combined dilute effect from high moisture content. Sometimes SSC values decline because the fruit develops a mealy texture. Fruit with low firmness often have lower SSC because the cells aren't broken open and don't release the sugars. In this work SSC did not decrease in pace with FFF, two independent process were implied.

Within first 20 days of storage, SOD activity of apples in all packagings increased after detachment from the tree and exposure to storage conditions. The high SOD could be another reason for ascorbic acid content being maintained during the first 40-day storage (Table 4). After another 40-day storage, SOD activity fluctuated at low levels, implying its antioxidant protection became sustaintially weaker.

Sandhya (2010) reported MA containing < 8% O_2 and > 1% CO_2 retarded Fuji apple fruit ripening. This study supports Sandhya's conclusion because only mPVC bags, which elevated CO_2 to more than 2% (2.0 kPa), delayed fruit decay and disorder development through 220-day storage. Control-1 is applied popularly in storage industry as a cheap MA packaging. It actually developed no gaseous-MA condition according to the measurement in this study; however, it resembled mpPE on reducing superficial scald significantly compared to non-MA packaging (control-2), though higher scald than mPVC. Its advantage over control-2 in less fresh weight loss as well as higher SSC maintenance may work as a function of moisture-MA, enabling it control some disorder of apple. Thus, MA means proper modified CO_2, O_2 and moisture; the more conditions are satisfied, the better storage result will be achieved.

Core browning of apple fruit was found to be induced by low endogenous O_2 (<1.6%) (Yeardley *et al.*, 1997) or high CO_2 (10%) (Guan *et al.*, 2004). Wang *et al.* (2002) also found that >5% CO_2 induced core browning, however, core browning could result from fruit senescence as well. In the current study, mPVC bags resulted in the highest CO_2 and lowest O_2 concentrations among four MAPs and also the least core browning, which implies that core browning was more due to senescent processes than MA conditions.

For a 0-1 °C storage as long as about 7 months, polyolefin bags made of mPVC maintained Fuji fruit in best quality. The fruit developed no decay or disorder after 220-day storage and only had among the lowest decay, the least superficial scald and core browning after 270-day storage. The maximum storage life of Fuji apples is likely around 220 days in mPVC bags at 0-1 °C because scald and core browning accelerated thereafter. For a 0-1 °C storage shorter than 6 months, PFM (control-1) bag is suggested the preferred packaging for Fuji apple because similar results as that from mPVC bag could be obtained at a lowest cost. PFM showed no gaseous-MA effect, but proper moisture-MA function, which benefited the partly control of superficial scald of the apple. The combined effects of reduced ethylene production and respiration resulted from an effective MA delayed senescence and resulted in the overall better fruit quality after storage.

Acknowledgement

This work was funded by the project 'National Technical System for Agricultural Industry' financed by the Ministry of Agriculture of China.

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Received: March, 2010; Revised: June, 2010; Accepted: July, 2010

Journal of Applied Horticulture, 12(2): 140-144, July-December, 2010

Horizontal and vertical soilless growing systems under Cyprus conditions

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Abstract

Under the impact of new cultivation and socioeconomic trends, and the aspiration for agricultural sustainability, a research study was conducted under Cyprus conditions. Lettuce (*Lactuca sativa* cvs. 'Paris island', 'Lollo rosa', and 'Oakleaf') and strawberry (*Fragaria* x *ananassa* cv. 'Camarosa') plants were used to evaluate horizontal and vertical growing setups in a 'closed' soilless system. For lettuce, the vertical system provided more marketable lettuce per system's surface area compared to the horizontal setup. However, the horizontal system provided greater lettuce mass and higher percentage marketable yield than the vertical one. The nitrate content of all lettuce cultivars was not significantly different between the two systems and remained lower than the European standards all over the experiment. For strawberry, the vertical setup offered higher yield compared to the horizontal one. The quality characteristics were not different between the two systems. These results suggest that the studied setups and the 'closed' soilless system can be used as a tool for the improvement of Cyprus greenhouse production, water use efficiency and prevention of environmental damage from regular` disposal of hydroponics solution. The possibility of an improved greenhouse production system could be considered as technique of choice under semi-arid Cyprus and E. Mediterranean conditions using such materials.

Key words: Lettuce (*Lactuca sativa*), strawberry (*Fragaria x ananassa*), horizontal system, vertical system, hydroponics

Introduction

Achieving agricultural sustainability is a major goal in many parts of the world, primarily in the developed countries. The increased environmental awareness, the need to protect the various ecosystems, as well as the ongoing effort to implement agricultural practices that are economically viable and environmentally sound, give an impulse for the use of soilless growing techniques and a new research directive. Consequently, the use of soilless systems in North America, the European Union and the Middle East is popular, not only because of the preceded trends, but as a natural consequence of the climatic conditions of these regions. Naturally, the closed hydroponic systems gain familiarity, because of the water scarcity in parts of the world, and the legislation demanding the adoption of closed hydroponic systems in many countries (Avidan, 2000; De Kreij *et al.*, 2001). However, 'closed' systems are seldom completely closed, because some ions or chemicals will eventually build up to an excessive level, and generally, it is less expensive to discard such a solution than to clean it (Schröder and Lieth, 2002).

In Cyprus, few studies have been conducted targeting the hydroponic production of cut flowers and vegetables (Chimonidou, 1999, 2003; Polycarpou *et al.*, 2005; Neocleous *et al.*, 2005, 2007). However, it is necessary to conduct further studies, because of the new cultivating and socio-economic trends, and the pollution of the surface and subsurface waters with nitrates from fertilizers. Furthermore, there is an increasing interest in soilless culture and vertical systems, because it provides better energy utilization and more efficient use of the greenhouse volume, resulting in higher yield per unit area (Paraskevopoulou-Paroussi and Paroussis, 1995). Eventually, these studies will increase the competitiveness of the Cyprus hydroponic production, and help the local agricultural community adopt the framework of the Environment Action of the European Community (EC Decision No. 1600/2002) and follow the Code of Good Agricultural Practices (Cyprus Government, Decision 407/2002).

The aim of this work was to study the feasibility of horizontal and vertical growing systems, with the subsequent minimization of nutrient solution losses to the environment, under Cyprus (E. Mediterranean) conditions. To achieve the latter, a closed hydroponic system was used and quantitative assessment of the two systems´ production was conducted.

Materials and methods

The evaluation of the horizontal and vertical systems was done in a polyethylene-coated, omega type triple-span greenhouse, 27 m long, 21 m wide and 5 m high with side and roof openings, and a 'closed' hydroponic system, at the Zygi Experimental Station of the Cyprus Agricultural Research Institute (long. 32°E, lat. 35°N) during late Winter - early Summer in 2006. All systems were built on a pilot-scale in order to provide the fundamental data for further investigation and future large-scale implementation.

Description of the horizontal and vertical systems: Sixteen canals for substrates (Ramat Hashofet, Israel), with dimensions 0.17 x 0.20 m and length 11 m each, were placed 40 cm apart within the greenhouse, to serve as the horizontal system's growing setup. In order to prevent the formation of algae due to the presence of photosynthetic microorganisms, black nylon and black-ash wood covered the connections between the canals.

A computerized time-control irrigation unit (Macqu) controlled the electrovalve of the greenhouse, monitoring the pH and the EC of the irrigation solution. Polyvinyl chloride (PVC) 16 mm inline drip irrigation tubing, with drippers of 4 L h^{-1}, spaced 30 cm apart, was used to deliver the nutrient solution to the plants, and the drainage solution was collected in a reservoir for reuse. For the vertical system, eighty grow-bags were used (Sunsaver, Spain), with height 1.80 m, and 12 sacks (6 on each side). Each sack's dimensions were 12 x 8 cm. To support the grow-bags in two rows of forty bags, two 1.91 cm diameter galvanized steel pipes were installed at a height of 2.50 m, parallel to the ground, and spaced 1.50 m apart. The distance between each grow-bag was 10 cm, and Macqu controlled the irrigation. For the irrigation of the vertical system, a 16 mm PVC inline drip irrigation tubing (spaghetti) with pairs of 25 cm spaced emitters was used providing a total of 16 L h^{-1} for every grow-bag. With the aid of a black nylon canal, under every row of grow-bags, the drainage solution was collected for reuse. A UV lamp (Montagna) disinfested the drainage solution of both systems, prior any reuse, in order to prevent the development of any microbial pathogens.

Irrigation practices: An experimental 'closed' system using software developed in an earlier EU-project (MACQU, AIR 1603) was used. A central computer was utilized to send the desired functional and control parameters to stations (setup), and also to provide the user with an image of the greenhouse. The automatically started irrigation time program operated every day from 06:00 to 18:00. In each day we defined up to 10 time intervals during which we wanted the program to operate. Irrigation was done through a drip irrigation system. The amount of the water applied was estimated according to the amount of drainage solution (30-40%). The drainage solution from the substrates was first filtered (150 μm) and then disinfected through the UV lamp.

Definition of the hydroponics parameters: The EC and pH of the solution were regulated using Macqu as mentioned previously. The desired pH and the lower and upper pH limits were determined for the solution according to basic nutrient solution concentrations (see below). In case that the pH was under the lower limit, the program closed the irrigation valves and opened the reject valve so that fresh water entered in the mixing tank. If the pH was higher than the upper limit, the program closed the irrigation valves and tried to reduce the pH by adding acid in the mixing tank. The desired electrical conductivity (EC) and the lower and upper EC limits were specified for the solution. In case that the EC was under the lower limit, the program closed the irrigation valves and added condensed solutions in the mixing tank. If the EC was higher than the upper limit, the program closed the irrigation valves and opened the reject valve so that fresh water entered the mixing tank to lower the EC. Such a system minimizes the build up of toxic ion concentrations in the solution. As a consequence it was not necessary to discard the solution more often than 2 months. The substrate for both systems was 100% hydroponic perlite. Moreover, a greenhouse environmental control unit, Galileo Greenhouse irrigation and climate control V_2 (Eldar, Shany), controlled the automatic opening of the windows at 20°C, the closing of the windows and the start of the cooling system (cooling pad and fans) at 25°C, while maintaining a desirable range of relative humidity between 40 to 65%. The nutrient solution consisted of (ppm): N (127.6), P (36.6), K (181), Ca (89.9), Mg (42.89), S (36), Fe (0.85), Mn (0.250), Cu (0.01), B (0.25), Zn (0.125), and Mo (0.025). The iron was in the form of Fe-EDDHA chelate. The corresponding pH and EC values for the solution were 6.2 and 1.25 mS cm^{-1}, respectively.

Pest management: Insects and diseases were controlled using Integrated Pest Management (IPM) techniques. Pests were controlled successfully with a combination of: a) mechanical control using fine-mesh screens on the windows, a soil-cover to prevent the pupae stage of various insects, and a double door entrance hall with good insulation; b) physical control using yellow traps (chromo-attractive control) painted with glue and hung between the plants of both systems, as well as monitoring and scouting (24 plants were selected to be observed); c) biological control using beneficial insects such as the predatory bug *Orius laevigatus* and the biologically-derived insecticide Tracer (Spinosad); d) chemical control with the acaricides Pride (Fenazaquin) and Nissorun (Hexythiazox). Sulphur fumigators worked every night from 2 am to 3 am in order to prevent mycological disease and control the populations of *Tetranychus* spp.

Plant transplantation: The experimental crops were strawberry (*Fragaria x ananassa*, cv. 'Camarosa') and lettuce (*Lactuca sativa*). The lettuce variety 'Paris island' was used during the first lettuce growing season, followed by 'Lollo rosa' and 'Oakleaf'. The seedlings were raised on peat compost cubes, and were transplanted in the horizontal and vertical system when 5-7 cm tall. In twelve canals of the horizontal system, 'Paris island' plants were transplanted (6 plants/m), and strawberries were transplanted in the remaining four canals (5 plants/m). Strawberries were placed into seventy-two grow-bags (12 plants/bag), and lettuce was placed in the rest eight bags (12 plants/bag).

General methodology: Collection of the environmental data of the greenhouse was achieved using a 21X Micrologger data logger (Campbell Scientific), two MP 100A temperature – relative humidity probes (Campbell Scientific), and a CM3 Pyranometer (Kipp & Zonen). Nutrient solution supply was recalculated on a regular basis using the readings of the applied and drainage water. IPM data such as population count in the monitoring traps and monitoring plants were gathered on a weekly basis. The experiment was a completely randomised design. Analysis of variance (ANOVA) and mean separation by Duncan's multiple range tests were performed on the lettuce and strawberry datasets using the SAS statistical software, Release 8.2 (SAS Institute Inc.).

Lettuce methodology: Sampling and analysis began two weeks after the transplantation. Randomised lettuce samples were analysed to determine their: a) leaf fresh weight, root fresh weight, leaf dry weight (%), and root dry weight (%) ('Paris island'); b) leaf fresh weight, and leaf dry weight (%) ('Lollo rosa' and 'Oakleaf'); c) nitrate and chloride content ('Paris island', 'Lollo rosa', and 'Oakleaf'). Fresh weight was measured after harvesting the upper part of lettuce above the basal area. The above samples were dried at 65°C to constant weight. For the potentiometric determination of the nitrate concentration in the dry sample, a nitrate-ion electrode and the pH & Conductivity meter EC30 (HACH) were used. Furthermore, for the determination of the dry sample's chloride content a titration with $AgNO_3$ was used.

Strawberry methodology: A random selection of strawberry plants was monitored for its a) auxiliary shoot production; b) fruit fresh weight, fruits picked per surface area, and non-marketable fruit quantity; c) vitamin C, pH, soluble solids concentration, titratable acidity, and total anthocyanin content.

Analyses: For ascorbic acid (vitamin C) determination randomised strawberry samples of 16 fruits, stored overnight at 4°C, were homogenized for 5 min. 0.5 g of homogenized sample was added to 40 mL extracting solution (oxalic acid 1%), homogenized for 1 min, and filtered through Watman (No. 1) filter paper. The ascorbic acid content was measured using the instrument RQflex Plus (Merck) in combination with a standard solution of known ascorbic acid concentration (200 ppm). Ascorbic acid reduces yellow molybdo-phosphoric acid to phosphomolybdenum blue, the concentration of which is determined reflectometrically. Ascorbic acid analytical test strips, Reflectoquant, were provided by Merck (Darmstad, Germany). The test strip was inserted into the sample and transferred into the RQflex Plus adapter for the measurement result.

The pH of the pulp was recorded by a high-impedance voltmeter AGB-4001 pH Meter (Labtech Instruments). Soluble Solids Concentration (S.S.C) at 20 °C was determined in the homogenized sample using the Automatic Refractometer GPR 11-37 (Index Instruments).

For the determination of the total anthocyanin content a modification of the Torre and Barrit method (1977) was used. 1 g of homogenized sample was added to 50 mL of extracting solution (ethanol 80% + HCl 2%). The mixture was stored for 24 h at 4°C and then filtered through Watman (No. 1) filter paper. Absorbance readings were determined at the maximum absorbance peak of 534 nm, after scanning the samples from 400 to 600 nm for maximum absorbance wavelength, using a M350 Double-beam UV-Visible spectrophotometer (Camspec) and quartz cuvettes with 5 cm optical path. The absorbance values were expressed as units of optical density per gram fresh weight [units OD (g fresh wt)$^{-1}$].

The titratable acidity was measured by mixing 2 g of the pulp and 50 mL distilled water with few drops of phenolphthalein indicator and titrating the mixture with 0.1 N NaOH. The titratable acidity was expressed as % citric acid equivalent to the quantity of NaOH used for the titration (Ryan and Dupant, 1973).

Results and discussion

Greenhouse data: The daytime temperature varied from 21.1 to 34.9°C, having a mean value of 26.8°C throughout the experiment. The average nighttime temperature had a mean value of 15.5°C, fluctuating from 8.3 to 19.7°C. During the daytime the average relative humidity was 52.4%, varying within the range of 21.5 and 65.9%, whereas the nighttime average relative humidity was 76.3%, with minimum and maximum values at 53.5 and 82.2%, respectively. The above measurements indicate the proper functioning of the climate control system, since all values were within an acceptable range, considering the warm climatic conditions of Cyprus during spring and summer. During the experiment, the intensity of the solar radiation varied from 355 to 1110 W m^{-2}, with a mean value of 867 W m^{-2}.

Generally, the nutrient solution was within the desirable working parameters as mentioned earlier, although sometimes the drainage pH increased significantly reaching a non-favourable pH mean value of 7.7 for strawberry cultivation. This increase could be justifiable because of the accumulation of catabolites/bicarbonates in the root environment, which washed out during leaching. Consequently, treating the drainage solution was essential, before reusing for the plants.

Pest management observations: Pest and diseases were controlled successfully by the means presented previously. Briefly, throughout the experiment, the major pests found were thrips and whiteflies. Their population rose up during May and this may be due to the higher temperature and humidity in the greenhouse. Thrips, whiteflies and leafminers were found on the lettuce of the horizontal setup, whereas on that of the vertical setup only thrips were detected. Thrips and mites were found on the strawberries of both systems.

Lettuce data: The leaf fresh weight of the horizontal system was much higher compared to that of the vertical setup, for all three lettuce cultivars (Table 1). However, this was not the case regarding leaf dry weight (%), where the vertical setup provided higher values. This may indicate the lower water intake and higher concentration of solutes by the vertical system's plants. On the vertical system, the fresh weight of the varieties 'Lollo rosa' and 'Oakleaf' diminished by ~45% whereas that of 'Paris island's' by 64%, possibly due to the excessive foliage and the bigger water needs. Comparing the root weight of 'Paris island', although there was no statistical difference in the root fresh weight, there was an increase in the root dry weight (%) similarly to the leaf dry weight.

The fresh leaf weight distribution along the grow-bag (Fig. 1A) for 'Lollo rosa' and 'Oakleaf' provided additional evidence for the water intake capability of the plants as a factor of their position. Therefore, the plants on the upper part of the bag with probably more nutrient solution availability had higher weights than the ones of the lower part. Although, also this trend hold for the leaf dry weight (%) of 'Oakleaf' (Fig. 1B), with the upper parts having more moisture content than the lower ones, there was no statistical difference in the leaf dry weight (%) of 'Lollo rosa'.

For lettuce, a crop with large water needs, the horizontal system provided bigger size and higher percentage of marketable yield than the vertical one in all the three cultivars (Table 1 and 2). Comparing the marketable lettuce (unit) per system's surface area, it was evident (Table 2) that although in horizontal system the percentage of the marketable quantity was higher; the vertical

Table 1. Lettuce plant fresh and dry weight in horizontal and vertical growing system for three cultivars

Cultivar	System	Leaf fresh weight (g)	Leaf dry weight (%)	Root fresh weight (g)	Root dry weight (%)
'Paris Island'	Horizontal	600a	3.40b	55.5a	9.62b
	Vertical	216b	5.71a	55.8a	11.6a
'Lollo rosa'	Horizontal	462a	3.86a	ND	ND
	Vertical	245b	3.90a	ND	ND
'Oakleaf'	Horizontal	428a	4.35b	ND	ND
	Vertical	241b	6.24a	ND	ND

ND: no data available. Means followed by the same letters within each cultivar in the same column are not significantly different, (Duncan's multiple range test $P \leq 0.05$)

Table 2. Marketable yield of lettuce cultivars in horizontal and vertical growing system (unit corresponds to one lettuce head)

Cultivar	System	Marketable quantity (%)	Yield (units m^{-2})
'Paris Island'	Horizontal	91.5	7.5a
	Vertical	64.6	31.0b
'Lollo rosa'	Horizontal	95.5	7.6a
	Vertical	77.9	37.0b
'Oakleaf'	Horizontal	92.7	7.34a
	Vertical	73.9	35.5b

Means followed by the same letters within each cultivar in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$).

Table 3. Nitrate and chloride concentrations of lettuce cultivars in horizontal and vertical growing system

Cultivar	System	NO_3 [mg (kg FW)$^{-1}$]	Cl (%)
'Paris Island'	Horizontal	445a	1.09a
	Vertical	375a	0.95a
'Lollo rosa'	Horizontal	2 277a	0.91a
	Vertical	2 033a	0.94a
'Oakleaf'	Horizontal	2 361a	0.97a
	Vertical	2 212a	0.69b

Means followed by the same letters within each cultivar in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$)

Table 4. Strawberry production characteristics in horizontal and vertical growing system

System	Fruit mass (g)	Fruits picked (units m^{-2})	Non-marketable fruits (units)	Auxiliary shoots (units)
Horizontal	15.3a	9.37a	0.53a	10a
Vertical	12.3a	21.8b	0.93b	4.25b

Means followed by the same letters in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$)

Table 5. Strawberry quality characteristics in horizontal and vertical growing system

System	pH	Ascorbic acid (vitamin C) (mg/100g FW)	Total anthocyanins [units OD (g FW)$^{-1}$]	Titratable acidity (% citric acid)	Soluble solids (% brix)
Horizontal	3.77a	39.7a	1.22a	0.79a	10.3a
Vertical	3.70a	44.2a	1.32a	0.82a	9.8a

Means followed by the same letters in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$)

Table 6. Strawberry flavour evaluation* in horizontal and vertical growing system

System	Size	Appearance	Firmness	Colour	Flavour	Aroma
Horizontal	4.3a	4.2a	3.8a	4.4a	4.3a	4.1a
Vertical	4a	4.3a	4.1a	4.3a	3.9a	4a

*Scale 1 (low) - 5 (high). Means followed by the same letters in the same column are not significantly different (Duncan's multiple range test $P \leq 0.05$)

system provided finally more marketable units m^{-2} for all cultivars, due to the better space utilization and compact setup.

Determination of the nitrate and chloride content of the lettuce cultivars from both systems indicated no differences in the NO_3 content (Table 3). For the chloride concentration, there were no differences, apart from 'Oakleaf', where the vertical plants had 0.69% Cl content compared to 0.97% of the horizontal system.

Strawberry data: Although the horizontal system produced slightly larger fruits (15.3 g) than the vertical system, however the difference was not significant (Table 4). However, the difference was significant regarding fruits picked, non-marketable fruits and auxiliary shoot formation between the two setups. There was a large difference in the number of fruits picked m^{-2} between the two systems (21.8 for the vertical and 9.37 for the horizontal), because of the larger population of strawberry plants in the vertical system (Table 4). This indicates that the vertical setup's produced higher yield due to better space utilization. Auxiliary shoot production was more than two times higher in the horizontal system (10 shoots per plant) compared to the plants in the vertical (4.25 shoots per plant) (Table 4). The lower auxiliary shoot production in the vertical system can be interpreted as fewer man-hours for the maintenance of the vertical setup's plants, compared to the pruning time needed for the horizontal plants.

There was no difference in fruit pH, ascorbic acid content, S.S.C. and titratable acidity between the two systems (Table 5). In accordance, strawberry flavour evaluation indicated no significant differences between the two systems (Table 6). Hence, no differences in flavour, aroma, colour, firmness, appearance, and size were found, indicating no consumer preference towards a specific cultivating system.

The vertical system under investigation performed well without any difficulties, providing higher marketable yield per system's surface area and proved to be suitable for the growth of crops like lettuce and strawberry. However, the horizontal system provided higher product fresh weight particularly in lettuce. The lower strawberry auxiliary shoot production in the vertical system can be interpreted as fewer man-hours for the maintenance of the

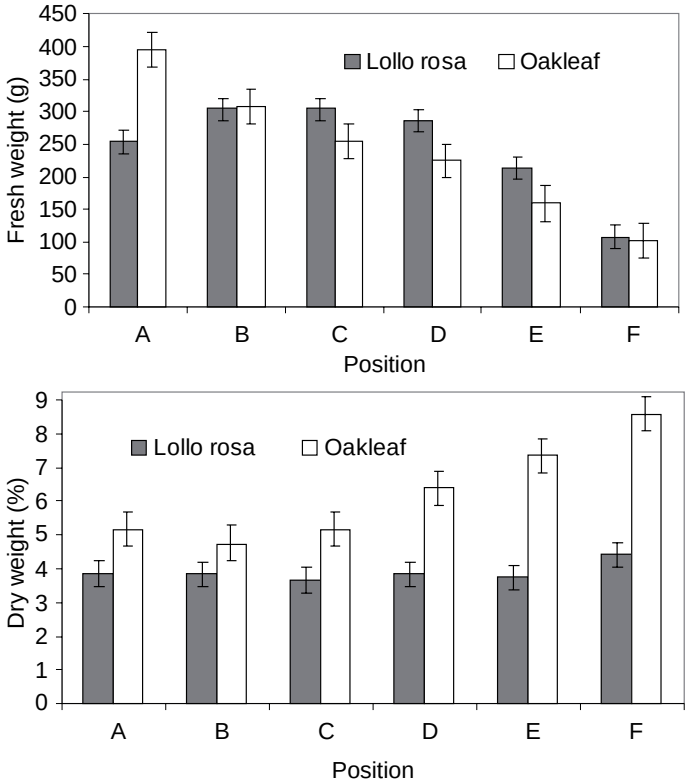

Fig. 1. (A) Fresh leaf weight distribution, and (B) dry leaf weight (%) distribution of 'Lollo rosa' and 'Oakleaf' along the grow-bag. Letters A through F indicate the sack's position, where A is the top sack and F the bottom one. SE bars are shown.

vertical setup's plants. For the strawberry, there was not any significant difference between qualitative characteristics for the two growing systems.

The obtained results suggest that the studied setups and the closed hydroponic system can be used as a sustainable system for the improvement of Cyprus greenhouse production, efficient water management and prevention of environmental damage from the frequent disposal of nutrient solution. Particularly, this system could be suitable for farmers with small greenhouse facilities. Moreover, it can be used in existing or future hydroponic facilities, particularly in areas proximal to vulnerable ecosystems, such as the rivers and coastline. Nevertheless, further studies should be conducted with other plants and substrates exploiting the system's capabilities for better adaptation by the agricultural community in the E. Mediterranean region.

Acknowledgement

This study was partially sponsored by the Cyprus Research Promoting Foundation (PENEK/ENTA 0509).

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Received: October, 2009; Revised: June, 2010; Accepted: October, 2010

Journal of Applied Horticulture, 12(2): 145-150, July-December, 2010

Improving yield and fruit quality of date palm by organic fertilizer sources

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Abstract

A field study was carried out during 2007 and 2008 seasons on twenty-six years old Zaghloul cultivar of date palm growing in clay silt soil. One level of nitrogen alone or plus P and K from mineral (ammonium nitrate alone or ammonium nitrate + calcium superphosphate + potassium sulphate, NPK) and organic sources [poultry /chicken manure (CM), cow dung (CD) and town refuse compost (TR)] were applied either alone or in combinations to study their influence on the yield and fruit physico-chemical quality. The results revealed that applying organic manure either alone or combined with mineral fertilizers increased palm yield and enhanced fruit colour as compared with mineral fertilization alone. CM and CD resulted in the best fruit weight, fruit flesh weight and length. Fruit TSS, anthocyanin and sugars content increased while, tannins content was decreased by CM and CD as compared with combining organic manure with NPK or mineral alone. However, fruit acidity was not affected by any of the treatments when compared among each others. In general, micronutrients contents were significantly higher in fruits by applying organic manure alone than organic manure combined with NPK or mineral fertilization alone. Organic manure fertilization alone (especially CM and CD) resulted in decreasing lead, cadmium, nitrate and nitrite content than mineral fertilization.

Key words: Organic, inorganic, fertilization, quality, Zaghloul dates, yield, poultry manure, cow dung, town refuse compost

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees in the world, known as tree of life because of its resilience, its need for limited water inputs, its long term productivity and its multiple purpose qualities. In Egypt, dates are an important traditional crop. According to FAO (2009), Egypt is considered as leading country among the top ten date producers (11,30000 tones). Zaghloul date is the most important soft cultivars grown in Egypt. It is usually harvested and consumed at the Khalal (Bisr) stage. Most of the date palms in Egypt are growing in loam and sandy loam soils. With time, these types of soils may become deficient in N, P, K, Mg and B (Tisdale and Nelson, 1978).

Date palm yield and fruit quality are mostly dependant on cultivar, nutrition and water relations. Fertilization programs play an effective role in increasing palm yield and improving dates quality. The use of chemical fertilizer is necessary for supplying the nutrient requirements. However, the continual use of chemical fertilization leads to deterioration of soil characteristics and fertility (Shimbo *et al*., 2001). Also, it is reported that chemical fertilizers such as super phosphate contain Cd and Pb and they may be the major source of cadmium uptake in plant (Shimbo *et al*., 2001). Thus, continuous use of chemical fertilizer might lead to the accumulation of heavy metals in plant tissues which contributes to fruit nutrition value and edible quality. The second source of nutrients is organic manure which is derived from animal or plant sources. It is an excellent source of organic matter and macro and micro-nutrients. Animal manure is an important source of N, P and K and its addition to the soil increases the available phosphorus and exchangeable K, Ca and Mg contents (Magdoff, 1998). In addition to providing nutrients for crop growth, manure has several beneficial effects on soil properties. Application of organic fertilizers improved structural stability and lowered bulk density of the soil, improved moisture retention, water infiltration rate and the hydraulic conductivity of soil (Tisdale *et al*., 1990; Young, 1997). Also, manures were found to enhance soil biological properties (Chai *et al*., 1988) and soil fertility leading to increase in crop yield (Lal and Mathur, 1989). Organic manure may be beneficial to crop and soil on the long term (Tirol-Padre *et al*., 2007), and their efficiency in enhancing crop growth and yield in the short term by combining them with mineral fertilizers has been reported (Kanal and Kuldkepp, 1993; Mottaghian *et al*., 2008).

In view of the above facts, the present study was undertaken to investigate the effect of different nutrient supply regimes namely, cow dung, chicken manure, town refuse compost and mineral fertilizer on the yield and fruit quality and nutritional value of Zaghloul date palm growing in clay silt soils and irrigated with drainage water.

Materials and methods

Plant material and experimental design: Field experiment was conducted during 2007 and 2008 seasons in a private orchard at El- Nubaria region, EL- Behera Governorate, Egypt on date palms (*P. dactylifera* L.) cultivar, Zaghloul planted at 10x10 m apart. The trees were growing in clay silt soil and irrigated with drainage water. Soil was well drained with water table 110 cm and pH 8. The physical and chemical characters of the soil are presented in Table 1. The palms were fertilized with inorganic and/or organic fertilizers either alone or in combinations. The inorganic fertilizer was ammonium nitrate (33.5 %N) + triple super phosphate, (46%P_2O_5) + potassium sulphate (48% K_2O). Organic fertilizer were poultry manure (chicken manure, CM), cattle manure (cow

Table 1. Physical and chemical characteristics of experimental orchard soil (average of 2007 and 2008)

Characters	Soil depth (cm)		
	0-30	30 -60	60 – 90
$CaCO_3$ (%)	13.65	11.78	13.45
EC (ppm)	507	702	1523
Texture	Clay	Clay	Clay
N (%)	0.26	0.16	0.08
P (ppm)	54	37	46
K (ppm)	0.24	0.51	1.7
Ca^{++} (meq L^{-1})	5.7	6.0	12.3
Mg^{++} (meq L^{-1})	2.9	2.4	4.2
Na^{+} (meq L^{-1})	2.6	2.7	7.1
Fe (ppm)	25	21	22
Zn (ppm)	13	8	14
Mn (ppm)	54	34	50
Cu (ppm)	14	10	11
HCO_3^- (meq L^{-1})	6	5.4	5.1
Cl^- (meq L^{-1})	1.9	2.8	9.7
SO_4^- (meq L^{-1})	3.4	3.7	11.6

Table2. Chemical analysis of organic fertilizers used (average of both seasons 2007 and 2008)

Organic fertilizers	Parameters (%)				
	Moisture	Organic matter	Total N	P_2O_5	K_2O
Chicken Manure (CM)	9.32	33.54	3.38	1.68	1.72
Cow Dung (CD)	22.98	47.69	2.25	1.11	1.21
Town Refuse (TR)	33.36	58.43	1.35	0.64	0.72

dung, CD), and compost (town refuse, TR). Chemical analysis (average of both years) of organic fertilizers used is presented in Table 2. Each treatment included 1000 g nitrogen (applied from inorganic or organic source alone, or in combinations) + 535 g K_2O + 500 g P_2O_5 (estimated from the average amount of K_2O and P_2O_5 determined in the three organic manures). In both years mineral fertilizer was added at three intervals; half amount in March, a quarter in May and the last quarter in August of both years, and organic fertilizers were applied once at the second week of December. Each mineral fertilized Palm only received 3 kg ammonium nitrate + 1.1 kg triple super phosphate +1.1 kg potassium sulphate. Female palms were selected similar in growth, vigour, height, pollen source and age (30 years old) and were subjected to the normal cultural practices carried out as usual used for date palms. Eight soil application treatments were arranged in a complete randomized design with five replicates (1 replicate = 2 palms) per treatment (*i.e.,* 5 x 8 = 40 palm). The treatments were as follows: (1) 3 kg ammonium nitrate + 1 kg triple super phosphate + 1 kg potassium sulphate (NPK mineral only); (2) 3 kg ammonium nitrate (N only); (3) 30 kg Chicken manure (CM); (4) 45 kg Cow dung (CD); (5) 75 kg town refuse (TR); (6) ½ NPK mineral + ½ CM (½ treat.1 + ½ treat. 3); (7) ½ NPK mineral + ½ CD (½ treat.1 + ½ treat. 4); (8) ½ NPK mineral + ½ TR (½ treat.1 + ½ treat. 5).

Yield estimation: The fruits were harvested at the end of September in both years and the average yield and bunch weight was recorded in kilograms. Samples of 50 fruits per each palm were randomly taken (as a sample for each replicate) to determine fruit quality characters and mineral content.

Fruit physical characters: A 15 fruit sample from each replicate was taken to determine fruit weight (g), length (mm), diameter (mm) and flesh weight (g). Also fruit colour was recorded for each fruit sample using a degree of colour intensity as follow: (1) = 100 % green, (2) = 25% red, (3) = 50% red, (4) = 75% red and (5) =100% red.

Fruit chemical characters: In a 15 fruit sample, the percentage of total soluble solids (TSS) was determined in the juice using hand refractometer. Acidity was determined according to A.O.A.C. (1995). Soluble tannins content per 100 g fresh weight was determined as mentioned by Abou Sayed-Ahmed *et al.* (1997). Anthocyanin content in the fruit peel was measured using spectrophotometer by the method of Fuleki and Francis (1968). Total and reducing sugars as percentage of fresh weight were determined according to A.O.A.C. (1995). Non reducing sugars were calculated by the difference between total and reducing sugars.

Fruit mineral content: A sample of 20 fruits for each replicate was washed with tap water, rinsed twice in distilled water, dried to a constant weight in air drying oven at 70 °C, ground and digested with H_2O_2 and H_2SO_4 according to Evanhuis and De Waard (1980). Total nitrogen was determined colorimetrically according to Evanhuis (1976). Phosphorus was determined colorimetrically by ascorbic acid method according to Murphy and Riley (1962). Potassium content was determined by flame photometer. Pb, Cd, Ca, Mg, Fe, Zn, Mn and Cu contents were measured using an atomic absorption spectrophotometer 305B. The concentrations of N, P, K, Ca and Mg were expressed as percent, while Pd, Cd, Fe, Mn, Zn, and Cu were expressed as parts per million (ppm) on dry weight basis. Fruit nitrate and nitrite contents were measured according to Chapman and Pratt (1961) and expressed as ppm on dry weight basis.

Statistical analysis: All data were tested for treatments effects by analysis of variance (ANOVA) using Statistical Analysis System (SAS Institute, 1989).

Results and discussion

Yield: The data of both years presented in Table 3 showed that palm yield was significantly higher by applying CM, CD, ½ NPK mineral + ½ CM, ½ NPK mineral + ½ CD and ½NPK mineral + ½ TR than NPK or N mineral alone and TR. Chicken manure resulted in the highest yield as compared with the other treatments in the first year only. Our results support earlier findings which indicated the importance of supplementing the organic matter with mineral fertilizers to increase yield of date palms (Al-Bakr, 1982; Bacha and Abo-Hassan, 1983).

Fruit physical and chemical characters: The data for fruit physical and chemical characters in both years are presented in Table 3 and 4. As for the physical quality, a significant enhancement in fruit colour was obtained in both years by applying CM, CD, TR and ½NPK mineral + ½ CM when compared with NPK mineral or N fertilization with no significant differences obtained among them. In addition, ½NPK mineral + ½ CD and ½NPK mineral + ½TR resulted in higher increase in fruit colour than N mineral alone. Average fruit weight of both years did not significantly differ among all treatments (except N mineral in both years). Applying CM, CD, TR and ½NPK mineral + ½ CD gave significantly higher fruit weight than N mineral alone in both years. Also, NPK mineral (in the second year), ½NPK mineral + ½ CM and ½NPK mineral + ½ TR (in

the first year) resulted in higher fruit weight than N mineral alone. Similarly flesh weight did not significantly differ among all treatments (except N mineral alone) in both the years. Moreover, in both years NPK mineral, CM, CD, TR and ½NPK mineral + ½ CD had higher effect on average flesh weight than N mineral alone without significant differences among them. In both years fruit length was significantly higher than N mineral alone by all treatments (except town refuse in the first year). In addition, application of chicken manure (CM) resulted in higher fruit length than N mineral alone (in both years) and NPK mineral, TR, ½NPK mineral + ½ TR (first season). Also, application of cow dung (CD) alone resulted in higher fruit length than ½NPK mineral + ½ TR in both years. Fruit diameter did not significantly differ between all treatments (except N mineral and TR) and NPK mineral in both years. However, NPK mineral gave fruit diameter than N mineral and TR treatments in both years.

In addition, the data of different chemical characters determined in both years showed that application of CM, CD, TR, ½NPK mineral + ½ CM, ½NPK mineral + ½ CD and ½NPK mineral + ½ TR did not significantly differ from each other and they (except ½NPK mineral + ½ CM and ½NPK mineral + ½ CD in the first year) resulted in higher total soluble solids content than NPK or N mineral treatments. All treatments showed no significant difference among each other in affecting fruit juice acidity content in both years. In addition, the application of organic fertilizers either alone or in combinations with the NPK mineral fertilizer resulted in higher fruit non-reducing and total sugars contents than mineral fertilization alone (NPK or N mineral alone) in both years. However, only the application of chicken manure or cow dung gave higher non-reducing sugars content than NPK mineral in the first year only, whereas, in the second year no significant differences among all treatments were obtained except that ½NPK mineral + ½ CD gave higher non-reducing sugars content than ½NPK mineral + ½ CM and N mineral treatments. Fruit peel anthocyanin content increased significantly by the application of organic fertilizers alone (CM, CD or TR) when compared with the mineral fertilizer treatments (NPK or N mineral alone) in the first year only. The cow dung fertilizer (CD) resulted in higher anthocyanin content than all organic manure fertilizers combined with NPK mineral in both years. In addition, the data obtained of both years showed no significant difference in fruit pulp tannins content among all fertilizer applications except for nitrogen alone mineral fertilizer (N mineral). The application of nitrogen mineral fertilizer alone (N mineral) resulted in significantly higher content than town refuse (TR), ½NPK mineral + ½ CD and ½NPK mineral + ½ TR (in the first year), and than all treatments (second year).

Table 3. The effect of organic and inorganic fertilization on the yield and fruit physical characters of Zaghloul dates in 2007and 2008

Treatments/ year	Parameters					
	Yield kg/ palm	Fruit colour	Fruit weight (g)	Flesh weight (g)	Fruit length (mm)	Fruit diameter (mm)
2007						
NPK mineral	182	4.6	33.03	31.68	6.3	2.92
N mineral	178	4.5	30.34	28.76	5.8	2.12
Chicken manure (CM)	208	5.0	34.87	32.94	6.8	2.74
Cow Dung (CD)	198	4.9	34.76	32.95	6.6	2.84
Town refuse (TR)	179	4.9	32.62	30.73	5.9	2.24
½ NPKmineral + ½ CM	193	5.0	32.74	30.67	6.4	2.84
½ NPKmineral + ½ CD	200	4.8	33.97	31.85	6.7	2.86
½ NPKmineral + ½ TR	197	4.8	32.44	30.40	6.1	2.55
LSD (*P*=0.05)	8.0	0.3	2.85	2.14	0.43	0.61
2008						
NPK mineral	170	4.7	35.68	33.83	6.9	2.83
N mineral	159	4.5	31.69	28.89	5.4	2.03
Chicken manure (CM)	189	5.0	35.64	33.97	7.2	2.98
Cow dung (CD)	184	5.0	36.27	34.69	7.4	3.02
Town refuse (TR)	166	4.9	34.94	32.56	6.7	1.84
½ NPKmineral + ½ CM	184	4.9	34.11	31.98	6.8	2.84
½ NPKmineral + ½ CD	186	4.8	36.03	33.86	6.8	2.90
½ NPKmineral + ½ TR	185	4.8	34.01	31.86	6.4	2.35
LSD (*P*=0.05)	13	0.2	2.68	1.97	0.84	0.60

Table 4. The effect of organic and inorganic fertilization on the fruit chemical characters of Zaghloul dates in 2007and 2008

Treatment/ year	Parameters						
	TSS (%)	Acidity (%)	Reducing sugars (%)	Non-Redu. sugars (%)	Total sugars (%)	Anthocyanin (mg /100 g f.wt.)	Tannins (%)
2007							
NPK mineral	24.95	0.40	17.01	5.06	22.07	16.26	0.15
N mineral	25.60	0.45	15.83	6.04	21.87	14.36	0.17
Chicken manure (CM)	28.95	0.42	19.14	6.98	26.12	20.25	0.13
Cow dung (CD)	29.10	0.46	19.63	8.35	27.98	22.72	0.11
Town refuse (TR)	28.94	0.56	21.06	5.06	26.12	19.78	0.09
½ NPKmineral + ½ CM	27.70	0.46	20.28	5.06	25.34	17.83	0.12
½ NPKmineral + ½ CD	27.40	0.48	20.09	4.78	24.87	18.90	0.10
½ NPKmineral + ½ TR	29.05	0.45	19.83	7.25	27.08	18.22	0.10
LSD (*P*=0.05)	2.16	N.S	1.86	1.74	1.68	2.87	0.07
2008							
NPK mineral	26.47	0.39	16.08	6.46	18.45	18.45	0.1 9
N mineral	23.66	0.44	15.18	5.11	16.34	16.34	0.23
Chicken manure (CM)	30.93	0.45	20.83	6.64	18.35	18.35	0.16
Cow dung (CD)	30.98	0.51	20.01	6.96	19.12	19.12	0.18
Town refuse (TR)	31.92	0.48	20.96	6.80	17.85	17.85	0.18
½ NPKmineral + ½ CM	32.27	0.46	22.71	5.16	14.02	14.02	0.16
½ NPKmineral + ½ CD	30.89	0.49	19.36	7.51	15.28	15.28	0.19
½ NPKmineral + ½ TR	31.25	0.44	22.18	6.35	14.25	14.25	0.06
LSD (*P*=0.05)	3.18	N.S	2.43	2.13	1.96	1.96	0.04

Table 5. The effect of organic and inorganic fertilization on fruit macro and micro-nutrients content of Zaghloul dates in 2007 and 2008

Treatments/ year	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
2007									
NPK mineral	1.02	0.09	0.69	0.61	0.39	55	35	37	5
N mineral	0.96	0.19	0.98	0.78	0.34	52	30	38	6
Chicken manure (CM)	1.23	0.11	0.78	0.63	0.47	66	41	40	7
Cow dung (CD)	1.01	0.12	0.80	0.59	0.42	68	45	43	7
Town refuse (TR)	1.12	0.15	0.97	0.59	0.45	57	44	39	9
½ NPKmineral + ½ CM	1.05	0.13	0.90	0.61	0.39	54	34	43	8
½ NPKmineral + ½ CD	1.08	0.10	0.81	0.58	0.46	52	41	40	8
½ NPKmineral + ½ TR	1.14	0.10	0.90	0.62	0.40	57	42	36	7
LSD (*P*=0.05)	0.11	0.04	0.21	0.16	0.08	6.0	6.0	6.0	2
2008									
NPK mineral	1.19	0.09	0.71	0.57	0.35	58	28	31	5
N mineral	1.03	0.13	0.91	0.68	0.35	50	29	33	4
Chicken manure (CM)	1.13	0.14	0.82	0.64	0.44	63	33	40	6
Cow dung (CD)	1.09	0.11	0.99	0.67	0.40	69	30	41	7
Town refuse (TR)	1.17	0.14	0.99	0.63	0.41	62	39	40	8
½ NPKmineral + ½ CM	1.19	0.17	0.80	0.77	0.40	65	40	38	7
½ NPKmineral + ½ CD	1.26	0.19	0.90	0.69	0.42	64	44	34	6
½ NPKmineral + ½ TR	1.07	0.13	0.81	0.68	0.44	60	38	36	8
LSD (*P*=0.05)	0.08	0.02	0.15	0.19	0.05	7.0	4.0	3.0	4

The above results indicated enhancement in fruit quality characters by the application of organic manures either alone or when supplemented with mineral fertilizers. These results are in line with those reported by Bacha and Abo-Hassan (1983) and Shahein *et al.* (2003). In addition, Al-Kharusil *et al.* (2009) reported the highest dry matter content of date fruits by combining NPK mineral fertilizer with organic peat. In our study the application of nitrogen by combination of mineral and organic sources gave better fruit characters than using mineral source alone. Similarly, Sharawy (2005) reported that the combined application of N through mineral and compost was effective in improving fruit quality of lime trees compared to using each source alone.

Fruit mineral content

The effect of the different fertilization treatments on fruit mineral content is presented in Tables 5 and 6.

Table 6. The effect of organic and inorganic fertilization on fruit Pb, Cd, nitrate and nitrite contents of Zaghloul dates in 2007 and 2008

Treatments/ year	Parameters (ppm)			
	Lead	Cadmium	Nitrate	Nitrite
2007				
NPK mineral	1.08	0.010	50	7.9
N mineral	1.17	0.018	51	8.6
Chicken manure (CM)	0.89	0.010	39	5.5
Cow dung (CD)	0.94	0.009	37	6.7
Town refuse (TR)	0.78	0.008	48	6.2
½ NPKmineral + ½ CM	1.09	0.013	58	7.5
½ NPKmineral + ½ CD	1.06	0.011	56	7.7
½ NPKmineral + ½ TR	1.12	0.010	57	6.9
LSD (*P*=0.05)	0.10	0.008	3.0	0.7
2008				
NPK mineral	1.01	0.009	54	6.5
N mineral	1.22	0.020	58	7.8
Chicken manure (CM)	1.00	0.012	40	5.9
Cow dung (CD)	0.98	0.010	38	6.3
Town refuse (TR)	0.89	0.010	36	6.0
½ NPKmineral + ½ CM	1.08	0.015	45	6.7
½ NPKmineral + ½ CD	1.11	0.013	48	7.9
½ NPKmineral + ½ TR	1.09	0.016	50	6.9
LSD (*P*=0.05)	0.13	0.005	2.0	1.3

Macro and micronutrients: The data presented in Table 5 showed that in the first year only chicken manure and ½NPK mineral + ½ TR increased fruit nitrogen content than NPK mineral, whereas, in the second year fruit nitrogen contents was significantly lower by adding N mineral, CD and ½NPK mineral + ½ TR than NPK mineral. Moreover, no significant difference was obtained between NPK mineral and, TR, ½NPK mineral + ½ CM and ½NPK mineral + ½ CD in both years. Phosphorus content was higher by N alone fertilization than NPK in both years. Moreover, in the first year only TR, ½NPK mineral + ½ CM resulted in significantly higher phosphorus than NPK mineral, whereas, in the second year all fertilization increased phosphorus content in comparison with NPK mineral alone. Potassium content increased by the application of nitrogen mineral alone (N mineral) and town refuse (TR) in both years, ½NPK mineral + ½ CM and ½NPK mineral + ½ TR (in the first year) and cow dung (CD) and ½NPK mineral + ½ CD (in the second year) as compared with applying NPK mineral alone. In addition, the data of both years showed no significant difference when chicken manure and cow dung fertilizers were applied either alone or combined with NPK mineral. Moreover, only mineral nitrogen alone (N mineral) in the first year and ½NPK mineral + ½ CM in the second year gave higher fruit calcium content than NPK mineral. In the first year, only cow dung (CD) increased magnesium content as compared with NPK mineral, whereas, in the second year all treatments (except N alone) increased magnesium content as compared with NPK mineral fertilization. In addition, cow dung application gave significantly higher iron content than NPK mineral in both years. Also, chicken manure (in the first year) and ½NPK mineral + ½ CM (in the second year) had significant higher iron content than NPK mineral. Fruit zinc content was significantly higher by application of CM, TR, ½NPK mineral + ½ CD and ½NPK mineral + ½ TR than NPK or N mineral in both years. Manganese content increased by cow dung (CD) and ½NPK mineral + ½ CM as compared with NPK mineral in the first year, whereas, in the second year all treatments (except ½NPK mineral + ½ CD) increased manganese concentration as compared with NPK mineral and N alone treatments. Fruit copper was increased by all treatments (except N alone) as compared with NPK mineral

in the first year only. The application of organic fertilizers either alone or in combination with NPK mineral did not significantly differ from each other with respect to fruit Ca, Mg, Fe, Zn, Mn and Cu contents in both years.

The general, increase in fruit mineral contents as a result of organic manures application in combinations with mineral fertilizers might be due to the enhancement of soil properties and soil fertility by organic soil amendments (Mathew and Karikari, 1995; Kaur *et al.*, 2005) which might lead to the increase of available nutrients and their uptake (Kanal and Kuldkepp, 1993). Moreover, similar increase in fruit mineral contents by organic manures alone or in combination with mineral source were obtained by Attala *et al*. (2003) working on Samany and Zaghloul date cultivars.

Heavy metals, nitrate and nitrite: The data (Table 6) indicated that lead content significantly decreased in fruits fertilized with the three organic manures (CM, CD and TR) alone as compared with NPK mineral (in the first year) and N alone (in both the years). In addition, the application of organic fertilizers alone resulted in lower lead fruit content than combining them with the mineral NPK fertilizer in both years. Moreover, data of both years showed that fruit cadmium content did not differ significantly when organic fertilizers were applied either alone or in combinations (except ½NPK mineral + ½ CM and ½NPK mineral + ½ TR in the second year) as compared with NPK mineral. No significant differences were obtained between organic fertilization alone and organic fertilization combined with mineral fertilizer (except that combining TR manure with mineral NPK resulted in higher cadmium than town refuse alone in the second year only). In general, using mineral nitrogen fertilizer alone gave the highest lead and cadmium contents in comparison with all other fertilizing treatments in both years.

Nitrate content decreased significantly when chicken manure and cow dung (in both years) and town refuse (in the second year) were applied than NPK mineral and N alone fertilization. Moreover, in both years, nitrate content was significantly higher when organic manures were combined with mineral NPK than organic manures alone. In addition, in the first year, all treatments gave significantly lower nitrite content than nitrogen alone treatment with no differences obtained among them, except that CM gave the lowest nitrite content when compared with all other treatments. In the second year, nitrite content was significantly decreased by organic fertilization alone when compared with mineral nitrogen alone (N mineral) and ½NPK mineral + ½ CD. Our results are in line with those of Attala *et al.* (2003). In our study, the application of organic fertilizers alone resulted in lower fruit contents of Cd, Pb, nitrate and nitrite than applying them in combinations with mineral fertilizer or mineral fertilizer alone. Shimbo *et al.* (2001) stated that chemical fertilizers such as super phosphate contained cadmium and lead and it can be the major source of their uptake in plant.

From the findings of present investigation, we conclude that applying organic fertilization to Zaghloul date palms improved fruit quality and also resulted in better nutritional quality of the fruit than the mineral fertilization as it lowered the amount of heavy metals, nitrate and nitrite contents as compared with mineral fertilizing only.

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Received: November, 2009; Revised: August, 2010; Accepted: September, 2010

Journal of Applied Horticulture, 12(2): 151-154, July-December, 2010

Effects of arbuscular mycorrhizal inoculation on growth performance of *Piper longum* L. (Piperaceae) under sterilized soil conditions

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Abstract

A green house study was carried out to investigate the effect of inoculation with four native arbuscular mycorrhizal fungi (AMF), *Glomus mosseae*, *G. fasciculatum*, *G. clarum* and *G. versiforme* on growth performance of a medicinally important plant "Long pepper" (*Piper longum* L.). Inoculation with all AMF species enhanced plant growth, however, significant variation in effectiveness of the four AMF species was observed in relation to both root and shoot growth. A significantly higher total biomass (0.84g/plant) was observed in *G. fasciculatum* and *G. clarum* inoculated plants. The performance of *G. fasciculatum*, *G. clarum* and *G. versiforme* were statistically on par to each other in increasing the chlorophyll content over the control plants. The root colonizing capacity of *G. fasciculatum* was found to be significantly higher, the next being *G. versiforme*.

Key words: Arbuscular mycorrhizal, *Piper longum*, total biomass, chlorophyll content, *Glomus*

Introduction

Since long time, long pepper has been used as a medicine in 'Ayurveda', the ancient Indian Science of Life. It is an important ingredient in Ayurvedic principles/preparations such as 'Trikadu' and 'Panchakolam'. Long pepper of commerce is the dried spikes of at least four different species of *Piper (P. chaba*, *P. longum*, *P. mullesua* and *P. peepuloides)*, out of which *Piper longum* L. is the most commonly used species. Fruits of *P. longum* known as "Pippali" in Sanskrit are used as carminative, liver tonic, abortifacient and for the treatment of joint pains. *P. longum* is also one of the main components used in formulations for the treatment of gonorrhea, menstrual pain, tuberculosis, respiratory tract infections and arthritic conditions. It is also used in combination with other digestive herbs for promotion of proper digestive, bowel movement. Decoction of the fruit is used extensively in acute and chronic bronchitis and the alcoholic extract of fruits shows the promising immunomodulatory and antitumor activity (Hullatti *et al.*, 2006). Besides the spikes, the roots also have medicinal value, which contains three alkaloids *viz.*, piperine, piperlongumine or piplartine.

India is the major producer, exporter and consumer of long pepper. The plant has an annual demand of 6280 tonnes (2004-05) with an annual increase in demand of 16.3% [National Medicinal Plant Board (NMPB) web site]. As a result, it has been included in the list of 32 prioritized medicinal plants by NMPB. The plant grows wildly in Western Ghats, North-East and Himalayan region. It is cultivated also in the states of Kerala, Andhra Pradesh and Maharashtra. The plant is commonly propagated through stem cuttings and grows well in the areas with hot, moist climate and sandy loamy soil with rich organic matter and good moisture holding capacity. One-year-old stem cuttings are planted in the well-prepared field and the first crop comes after 6 months of planting.

Due to its immense economic importance, the plant is being overexploited from the wild by indiscriminate fruit collection for trade resulting into its disappearance from wild habitat at an alarming rate. The disappearance of the plant from its natural habitat can be prevented by promoting large scale cultivation. However, the problems associated with large scale cultivation are lack of quality planting material, mortality in field, poor growth and yield. These problems can be overcome by application of efficient AM fungi as biofertilizer. These fungi form symbiotic relationship with plant roots and improve the nutrient uptake by the host plant from soil thereby stimulating their growth (Smith and Read, 1997). They also enhance rooting of stem cuttings, reduce transplantation shock of the seedlings (Singh, 2002), improve their resistance to environmental stresses (Augé, 2001; Bagyaraj, 1991) and protect the host plant from root pathogens (Jalali and Jalali, 1991). They also interact synergistically with other beneficial soil microorganisms such as nitrogen fixers and phosphate solubilizers (Bagyaraj and Varma, 1995; Jeffries,1987). Therefore, in the past few decades, these AM fungi have emerged as potential biofertilizers, a cheap, environment friendly alternative to expensive chemical fertilizers (Srivastava *et al.*, 1996). Though, growth enhancement of many agricultural and horticultural crops through application of AM fungi has been reported, very little is known about their potential to enhance the productivity of medicinal plants belonging to the genus Piper and particularly *P. longum*. Therefore, the present study was carried out to investigate the influence of different AM fungi on the growth performance of *P. longum* plant.

Materials and methods

A pot culture experiment was conducted in the green house of Rajiv Gandhi University, Arunachal Pradesh, Itanagar from July to October 2006. For AMF species belonging to the genus *Glomus* namely *G. mosseae* (GM)*, G. fasciculatum* (GF), *G. clarum* (GCl) and *G. versiforme* (GV) were taken for the experiment. For each AMF species, one inoculation bed was prepared in the green house. The bed was filled with autoclaved sand soil mix (2:1)

as the rooting medium. Each bed was inoculated with culture of individual AMF species by taking out 40 g of soil inoculum from AMF pure culture pots maintained by our lab and by distributing the propagules containing large numbers of spores/sporocarps and mycelium uniformly. The beds were 10 cm thick in which inoculum were placed as a layer at approx. 5 cm from the top surface of the bed so that the growing roots from each stem cutting pass through the inoculum layer. Large numbers of piper cuttings of uniform size were planted in the prepared inoculum beds for one month before being transferred to plastic pots for further growth for next three months. This was done to ensure that plants become mycorrhizal and five live replicates are kept maintained in the pots during the entire period of the experiment. Control plants were also grown in control inoculation bed (without AMF inoculation).

All the plastic pots (200 g capacity) were properly surface cleaned and filled with 200 g autoclaved sand: soil mixture (2:1) before transplanting the rooted stem cutting. Each seedling was transplanted along with rhizosphere soil without disturbing the rooting zone by making a small cylindrical hole in the centre of the pot. The experiment was a completely randomized block design with four treatments and five replications. Treatments consisted of plants inoculated either with (a) GM, (b) GF, (c) GCl or (d) GV. The soil used in the experiment was acidic with pH 5.5, organic carbon 1.54 -2.51 mg g^{-1} and available P 0.38-0.66 µg g^{-1}. Pots were maintained in the green house at a temperature of 22 ± 1°C with 12 h fluorescent illumination with 8000 lx light intensity, and water was supplied daily to maintain the soil moisture level close to field capacity. Plants were grown without any application of fertilizer or pesticides. After 90 days of growth period, the harvesting was carried out. The biomass production was recorded in the form of shoot length, shoot fresh weight shoot dry weight and root fresh weight and root dry weight and also the total biomass of the plant. Dry weight was determined after drying the shoot and root separately at 60 °C to a constant weight in hot-air oven for 48 hours. Percentage mycorrhizal root colonization was estimated following grid line intersect method (Giovanetti and Mosse, 1980) after staining the roots with Trypan blue (Philips and Hayman, 1970). The total leaf chlorophyll content was determined spectrophotometerically after extraction in 80% acetone (Arnon, 1949).

The statistical analysis for all plant and mycorrizal parameters was done by a one-way ANOVA. The fungi were ranked for each character and compared pair wise using Duncan's Multiple Range Test at 5% level of significance (Little and Hills, 1978).

Result and discussion

AMF colonization significantly increased the shoot length, shoot fresh weight, shoot dry weight (Table 1) root fresh weight, root dry weight and the total biomass of the plant (P<0.05) (Table 2). A significant increase in chlorophyll content of AMF inoculated plants was also observed over the uninoculated control plants (Fig.1 A).

Inoculation with *G. fasciculatum* resulted into the maximum shoot length (31 cm) during the growth period and it was significantly different (P<0.05) from all other AMF inoculation. The shoot length of uninoculated plants was only 7.3 cm. The next best inoculant was *G. versiformae*, (22.8 cm) and the performance of this species was similar to *G. mossae* (20.7 cm). However, in case of both shoot fresh weight and shoot dry weight, the maximum increase was observed in *G. fasciculatum* inoculated plants followed by *G. clarum*, but both were statistically at par with each other. The minimum increase of both shoot fresh weight and dry weight over the control plants (0.26, 0.07 g respectively) was observed in plants inoculated with *G. versiformae* and *G. mossae* (1.90, 0.38 and 1.82, 0.37 g, respectively).

Table 1. Effect of AM fungal inoculation on shoot length, shoot fresh weight and shoot dry weight of *P. longum* seedlings

Treatments	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Uninoculated	07.3 ± 0.519a	0.26 ± 0.023a	0.07 ±0.008a
G. mossae	20.7 ± 0.707b	1.82 ± 0.308b	0.37 ± 0.038b
G. fasiculatum	31.0 ± 1.083c	2.77 ± 0.307c	0.54 ±0.024c
G. clarum	16.0 ± 0.651d	2.09 ± 0.098cd	0.47 ± 0.020cd
G. versiformae	22.8 ± 0.704b	1.90 ± 0.282bd	0.38 ± 0.053bd
LSD at 5%	2.247	0.693	0.093

Numbers followed by the same letter within a column are not significantly different (P<0.05) by Duncan's multiple range test. Values are the means of five replicates ± standard error.

Table 2. Effect of AM fungal inoculation on root fresh and dry weight, and total biomass of *P. longum* seedlings

Treatments	Root fresh weight (g)	Root dry weight (g)	Total biomass (g)
Uninoculated	0.62 ± 0.033a	0.17 ± 0.018a	0.23 ± 0.019a
G. mossae	0.66 ±0.033a	0.20 ± 0.13a	0.57 ± 0.039b
G. fasiculatum	1.46 ± 0.130b	0.30 ± 0.019b	0.84 ± 0.041c
G. clarum	1.03 ± 0.048b	0.24 ± 0.018b	0.71 ± 0.031d
G. versiformae	0.77 ± 0.031a	0.18 ± 0.017a	0.55 ± 0.044b
LSD at 5%	0.2	0.042	0.102

Numbers followed by the same letter within a column are not significantly different (P<0.05) by Duncan's multiple range test. Values are the means of five replicates ± standard error.

G. fasciculatum inoculated plants also showed superior performance in case of both root fresh and dry weight (1.46, 0.30 g, respectively) in comparison to control plants (0.626, 0.17 g, respectively). The second best AMF was *G. clarum*, its performance was also statistically at par with the former one and followed by *G. versiformae* and *G. mossae,* respectively. On the other hand, the performance of *G. versiformae* and *G. mossae* was statistically similar with that of control plants in increasing both the root fresh weight and dry weight. All the AMF inoculants tested significantly increased the total biomass production over the uninoculated control plants. The biomass was enhanced more due to inoculation with *G. fasciculatum* (0.84 g) followed by *G. clarum*. The efficacy of *G. versiformae* and *G. mossae* was found to be statistically similar.

The total chlorophyll content of both inoculated and uninoculated plants has been presented in Fig. 1 (A). The total chlorophyll content of leaves differed significantly and the content was highest in plants inoculated with *G. clarum* (1.163 mg g^{-1}), which was followed by *G. fasciculatum* (0.983 mg g^{-1}) and *G. versiformae* (0.885 mg g^{-1}). However, these three species did not differ significantly in this respect. The least amount of chlorophyll content (0.387 mg g^{-1}) was observed in uninoculated plant.

Piper seedlings showed varied response to the inoculation of different AM fungi. It has been reported that the effect of mycorrhization on plant development is influenced both by the

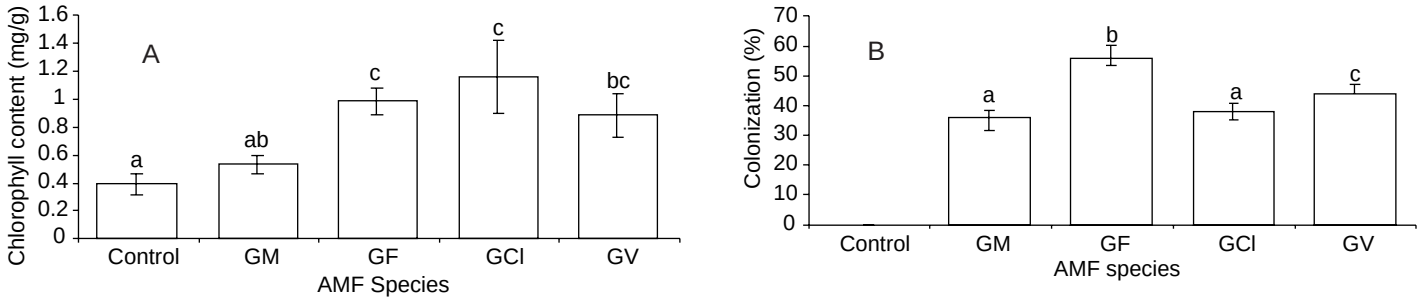

Fig. 1. Effects of inoculation with four different AM fungi on (A) chlorophyll content in plant leaves and (B) Colonization (%) in plant roots of *P. longum*. Histograms with a common letter are not significantly different (P<0.05) by Duncan's multiple-range test. Error bars indicate the standard error of the replicates

host plant and the fungal partner; hence different isolates of AM fungi can result in different effects on the same plant (Jackobsen *et al.*, 1992). Our study also supports this finding as all the four AMF species behaved differently in their growth promoting capabilities. Further, host preference among AM fungi has been reported by earlier researchers (Bagyaraj *et al.*, 1989; McGraw and Schenck, 1981). In present study, *G. fasciculatum* was found to be the most efficient AMF in increasing plant growth and at the same time it was also the most infective AMF colonizing the root cortical cells maximally. Therefore, it seems that this native AMF has some preference for the *P. longum* plant and their symbiotic relationship is most compatible. Enhanced plant growth due to mycorrhizal inoculation has been reported earlier in some medicinal plants also (Earanna *et al.*, 2002; Sailo and Bagyaraj, 2005; Sen and Das, 1998). This may be due to a combined effect of many processes contributing to improved P acquisition by mycorrhizal plants including increased absorption surface area of roots (Smith and Read, 1997) and increased exploration of soil micro sites by AMF hyphae (Cui and Caldwell, 1996). The enhancement of chlorophyll content due to AMF colonization has been reported by Morte *et al.* (2000). The increase of chlorophyll content in AM inoculated plant tissues may be due to an increase in stomatal conductance, photosynthesis, transpiration, enhanced plant growth (Hayman, 1983; Levi and Krikum, 1980), or due to the presence of larger or more numerous bundle sheath chloroplasts present in the inoculated leaves (Krishna and Bagyaraj, 1984).

The root colonization of *P. longum* by all four AMF species differed significantly (Fig.1B). Significantly maximum percentage root colonization was observed due to *G. fasciculatum* inoculation followed by *G. versiformae*. There was a positive trend between the intensity of mycorrhizal root colonization and growth response. This supports the observations made by earlier workers on other plants (Earanna *et al.*, 2002; Gracy and Bagyaraj, 2003; Kormanik *et al.*, 1982).

On the basis of the findings, the study suggests that AMF inoculation can effectively increase the growth of *P. longum*. Based on the growth performance of plants, *G. fasciculatum* was found to be the best AMF for inoculating *P. longum* followed by *G. clarum*.

Acknowledgements

The authors are thankful to the Department of Biotechnology, New Delhi for providing fund for this study and also the Department of Botany, Rajiv Gandhi University for providing the essential laboratory facilities to carry out the experiments.

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Received: November, 2009; Revised: July, 2010; Accepted: September, 2010

Journal of Applied Horticulture, 12(2): 155-157, July-December, 2010

Chemical composition and larvicidal activity of the essential oil of Iranian *Laurus nobilis* L.

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Abstract

The chemical composition of the essential oil obtained from the aerial parts of *Laurus nobilis* L., was examined by GC and GC/MS. The main components of the oil were identified. 1,8-cineole was the major component in the oil together with α – terpinyl acetate, terpinene – 4 – ol, α – pinene, β – pinene, p – cymene, linalool and terpinene – 4 – yl – acetate. The essential oil was tested against *Anopheles stephensi* and *Culex pipiens* larvae. The results obtained show that the essential oil could be considered as natural larvicidal agents.

Key words: *Laurus nobilis* L., essential oil, hydro distillation, larvicidal activity

Introduction

Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria, Japanese yellow fever, dengue and filariasis. The various synthetic products and devices designed to combat such vectors are not successful because of increased resistance developed by various mosquito species. Most of mosquito control programs target the larval stage in their breeding sites with larvicides, because adulticides may only reduce the adult population temporarily (El Hag *et al.,* 1999, 2001). The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent (Sukumar *et al.,* 1991).

Plant essential oils, in general, have been recognized as an important natural resource of insecticides (Gbolade *et al.,* 2000; Adebayo *et al.,* 1999). Their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects (Nishimura, 2001). They have the potential of being acute ovicidal, fumigant, insect growth regulator and insecticidal against various insects species (Tsao *et al.,* 1995) and concurrently being developed as ecologically sensitive pesticides (Isman, 2000). Generally, they are safe to humans and other mammals (Tripathi *et al.,* 2000, 2002).

As a part of our studies on the chemical composition of the essential oils and screening programme for bioactive compounds from plants that grow in Iran, in the present paper, we report the larvicidal activity of the essential oil obtained from the aerial parts of *L. nobilis* L. (Lauraceae) against two species of mosquito vectors, *A. stephensi* and *C. pipiens*. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

Materials and methods

Plant material: The aerial parts of *L. nobilis* L. were collected during its flowering stage from Tabriz (East Azerbaijan province, Iran) and identified. A voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Mosquitoes: The third instar larvae of *A. stephensi* and *C. pipiens* were obtained from laboratory bred culture maintained at ambient rearing conditions. All the bioassays were conducted at 26 ± 1 °C, 60.0 ± 5% RH and 12 h light and 12 h dark photoperiod. Yeast suspension (5%) was used as food source.

Isolation of the essential oil: Air-dried plant material (100 g) was hydro distilled for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and then was kept in a sealed vial at 4 °C until analysis.

Analysis of the essential oil: Gas chromatography analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The carrier gas was helium with a flow rate of 2 mL min^{-1}, the oven temperature for first 4 min was kept at 60 °C and then increased at a rate of 4 °C min^{-1} until reached to the temperature of 280 °C, injector and detector temperature were set at 280 °C.

The mass spectra were recorded on a Hewlett Packard 6890 MS detector coupled with Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The gas chromatography condition was as above. Mass spectrometer condition was as follows: ionised potential 70 eV, ionisation current 2A, source temperature 200 °C, resolution 1000, scan time 1 s.

Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. Quantitative data was obtained from FID area percentages without the use of correction factors (Adams, 1995; Dawis, 1990).

Larvicidal bioassay: Bioassays were performed according to the WHO protocol (WHO, 1981). A series of concentrations ranging from 2 to 100 μg mL^{-1} of the dissolved oil (in DMSO) was prepared and five replicates were run for each concentration.

Control tests were carried out in parallel, using DMSO and water for comparison. Malathion, a conventional insecticide was used as positive control sample. The number of dead larvae were counted after 24 h of exposure and the percentage mortality is reported from the average of five replicates. Observations were also made on the behaviour of larvae.

Statistical analysis: Probit analysis (Raymond *et al.,* 1993) was conducted on the mortality rate to determine the LC_{50} and LC_{90} representing the concentrations in µg mL^{-1} that caused 50% and 90% mortality along with 95% confidence limits.

Results and discussion

The hydrodistillation of aerial parts of *L. nobilis* gave 2.1 % (w/w) oil yield, based on the dry weight of the plant, that was yellow with distinct sharp odour. Twenty-two components were identified representing 99.5% of total oil. The qualitative and quantitative essential oil composition is presented in Table 1, where compounds are listed in order of their elution on the DB-1 column. The volatile compounds in aerial parts of *L. nobilis* mainly consisted of mono- and sesquiterpene hydrocarbons and their oxygenated derivatives. Besides phenolic compounds, also sesquiterpene lactones derived from the germacranolide costunolide was be found. As seen from Table 1, 1,8-cineole is the major component (55.8%), followed by α-terpinyl acetate (15.1 %), terpinene-4-ol (5.3 %), α-pinene (5.2 %), β-pinene (4.0 %), p-cymene (2.7 %), linalool (1.4 %) and terpinene-4-yl-acetate (1.1 %). In the present study, the chemical composition of the oil is comparable to that of the previous reports with some variation in the constituents. The observed chemical variations in the composition of the essential oil obtained from the same species are not uncommon. This could be due to different chemotypes for the same species or may result from environmental, developmental or other differences (Hafizoglu *et al.,* 1993; Kilic *et al.,* 2004).

The essential oil was subjected to laboratory bioassay studies against *A. stephensi* and *C. pipiens* larvae. The tested essential oil demonstrated significant larvicidal activity on both the vector species. Table 2 summarizes the LC_{50} and LC_{90} values for the essential oil. 1,8-cineole alone did not show promising activity in the dose re-sponse bioassay against any of the test larvae (mortality >50% was observed only at the highest testdose). Malathion (used as positive control) caused 100% mortality against all the larvae at very low test dose (>0.625 mg L^{-1}).

Table 1. Essential oil composition of the aerial parts of *L. nobilis* L.

Compounds	RT	KI	Percent
α- thujene	10:00	936	0.46
α- pinene	10:15	942	5.26
camphene	10:42	953	0.59
sabinene	11:27	972	3.42
β- pinene	11:37	976	4.06
α- terpinene	12:59	1010	0.50
p-cymene	13:05	1013	2.70
1,8-cineole	13:25	1021	55.80
γ-terpinene	14:28	1048	0.91
terpinolene	15:36	1077	0.35
linalool	15:45	1080	1.40
pinocarveol	17:15	1120	0.48
pinocarvone	17:49	1134	0.35
terpinene -4- ol	18:42	1158	5.27
α-terpineol	19:05	1168	0.85
bornyl acetate	22:40	1265	0.76
terpinene-4-yl acetate	23:43	1295	1.13
α- terpinyl acetate	24:54	1328	15.14
β- elemene	26:50	1382	0.15
β- caryophyllene	27:52	1412	0.15
spathulenol	32:34	1558	0.15
caryophyllene oxide	32:47	1564	Trace

Table 2. Larvicidal activity of essential oil from *L. nobilis* L. against *A. stephensi* and *C. pipiens*

Species	LC_{50} (µg mL^{-1})	LC_{90} (µg mL^{-1})	Regression equation	RP
A. stephensi	14.9	22.3	$y = 3.17x - 2.69$	0.076
C. pipiens	16.5	28.6	$y = 3.49x - 2.83$	0.078

All means are statistically significant ($P < 0.05$).
RP—Relative potency (LC_{50} standard/LC_{50} test substance).

The present study indicated that the essential oil from aerial parts of *L. nobilis* L. possessed remarkable larvicidal properties and compared favourably with the commercially available insecticide malathion. Larvicidal activity of 1,8-cineole, a major constituent (55.80 %) in the *L. nobilis* L. essential oil, was also studied to compare its activity with that of the *L. nobilis* L. oil. Surprisingly, this compound when tested alone failed to produce promising activity against any of the mosquito larvae (mortality >50% was observed only at the higher test dosages). In the present study, besides 1,8-cineole there are many other oxygenated monoterpenes and related compounds present in the oil. Thus, the activity of the oil against the mosquito larvae may be attributed to the additive or synergistic or blend effect of many or some of the constituents. Such an effect has been previously observed with some essential oils where the activity was due to the combination of the major constituents, none of which was found to exhibit significant activity, individually (Omolo *et al.,* 2005).

Recently, promising larvicidal activities of many essential oils and their compositions against mosquito vectors have reemphasised the need to explore the possibility of using essential oil-based products as supplementary and complimentary measures for mosquito borne diseases. Further studies are needed to devise a formulation using the oil and the compounds of this plant for use as larvicides in mosquito control programs.

Acknowledgement

We are grateful to the Faculty of Pharmacy, Tehran University of Medical Sciences, for the financial support to this investigation.

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Received: November, 2009; Revised: June, 2010; Accepted: July, 2010

Journal of Applied Horticulture, 12(2): 158-160, July-December, 2010

Evaluation of different substrates on yield and fruit quality of sweet pepper using open soilless culture

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Abstract

This study was conducted at Jordan Valley to evaluate the use of locally available tuff and sand substrates in comparison with soil for growing sweet pepper (*Capsicum annum* L. cv. Reehan) using an open soilless culture. Treatments were randomly distributed according to RCBD with three replications. Sweet pepper plants, grown in soil or tuff gave higher total yield (6.0, 5.5 and 8.7, 6.5 ton/1000m^2, respectively) and yield/plant (2.0, 1.58 and 1.3, 1.38 kg/plant, respectively) in both the years, while those grown in sand produced the least. Fruit weight of plants grown in soil was the highest in the first season (200.6 g) followed by tuff and lastly the sand (177.0 and 169.4 g, respectively), however, it was not affected by the substrates in the second season. Substrates had little effect on fruit length in both seasons and fruit diameter in the first season, but, in the second season those grown in soil gave the highest diameter (74.4 mm) followed by those in tuff and sand (70.6 and 70.3 mm, respectively). This study indicated that open soilless system using tuff as a substrate may be suitable for sweet pepper production without dramatic changes in yield or fruit quality and it saved about 65-70% of water applied by conventional farmers for sweet pepper production under plastic house.

Key words: Tuff, sand, soil, sweet pepper, soilless, fruit quality, yield

Introduction

Due to the arising problems of soil and the shortage of water supply for irrigation in Jordan, several farmers are using closed soilless cultures with the non-organic volcanic tuff as a substrate for production of cash crops such as cut flowers. However, the main disadvantage of such systems is the high initial establishment cost (Cooper, 1975; Winsor and Schwarz, 1990), therefore, the The National centre for Agricultural Research and Extension (NCARE) has developed a simple, cheap and effective open soilless culture to conduct this study at the Jordan Valley using locally available substrates to reduce the cost of imported ones.

In substrate culture, the nutrient solution can be applied in open or closed systems. In open systems, the nutrient solution is not recirculated, while it is recirculated in closed systems (Alan *et al.*, 1994; Çelikel, 1999; Cooper, 1975; NeSmith and Duval, 1998; Siomos *et al.*, 2001; Winsor and Schwarz, 1990). Under different substrates study, no significant difference was found between open and closed systems with respect to sweet pepper total yield (Tüzel *et al.*, 2001). Sand culture is one of the most efficient and a cost-effective method of soilless cultures due to its relatively low construction cost, simplicity of operation, ease of maintenance and service. However, sand culture requires sterilization between crops and feed lines may be blocked with sand particles, in addition to rapid salt build-up (Wright, 1992). Tuff culture has been used to grow several vegetable crops and cut flowers (Çelikel and Çaglar,1999; Economakois and Krulji, 2001; Hurewitz and Janes, 1983; Martin-Closas and Recasens, 2001; Tüzel *et al.*, 2001; Tüzel *et al.*, 2003). No significant difference was observed among different substrates with respect to bean total yield (Tüzel *et al.*, 2003), additionally, when sweet pepper plants were grown in different soilless substrates, fruit quality was comparable to those grown in soil culture (Çelikel and Abak, 1996). However, under greenhouse conditions, tuff resulted in higher tomato yield than soil (Abak and Çelikel, 1994).

This study was carried out to evaluate two locally available soilless substrates (tuff and sand) in comparison with conventional growing in soil for the growth of sweet pepper in a non-circulating open culture.

Materials and methods

This research was conducted at Wadi Al-Rayyan, northern Jordan Valley (200 m below sea level) during the 2001 and 2002 growing seasons. Treatments were tuff culture, sand culture and in soil, therefore, unheated plastic house was divided into six rows (two rows for each treatment). Soilless beds (40 cm wide, 30 cm deep and 10 m long) were made in soil with cement blocks and the ground was zero leveled. Each bed was lined with a 400-µ black polyethylene sheet to preserve the nutrient solution. Acid-washed sand and tuff were placed in the beds with equal volume in the first season. In the second season, the same substrates were re-washed with acid in the same beds**.** Tuff was placed in the beds in two layers; 5 cm of coarse tuff (8-16 mm in diameter) above it 15 cm of fine tuff (0-4 mm in diameter) was placed. The upper side of beds was covered with black plastic mulch, and an empty space was made at the end of soilless beds to monitor and control the nutrient solution.

A complete Hoagland's nutrient solution (Hoagland and Arnon, 1938) containing all macro and micronutrients was added to soilless beds manually (as needed) in a great caution since no drainage was available for excess solution. The level was kept between 5-15 cm according to the growth stage and its volume was recorded. The nutrient solution was daily monitored for EC

and pH and adjusted to 2.0-2.5 dS/m, and 5.5-6.0, respectively.

Soil beds were solar-sterilized, prepared, irrigated and fertilized as practiced by pepper farmers in the area of the study. Soil samples were collected from soil beds prior to planting and analyzed and contained Na 11 (meq/L), Mg 15 (meq/L), Ca 15 (meq/L), K 971 (ppm), P 20 (ppm), pH 7.5, EC 3.5 (dS/m) and $CaCO_3$ 37.9 (%). The total volume of water applied to soil was recorded using water meter. For all treatments, sweet pepper cv Reehan seedlings were planted on October 15th in double rows with 40 cm spacing.

At each harvest, fruits were collected; counted and weighed to determine total and marketable yields, yield per plant and average marketable fruit weight, length, diameter and skin thickness. At the end of the study, whole plants were collected, weighed and oven-dried to determine plant dry weight and N, P, and K content (A.O.A.C., 1970). Air temperature at 50 cm above ground and soil, sand and tuff temperature at 10 cm depth were measured with a data logger and recorded.

Treatments were randomly assigned the experimental units in a Randomized Complete Block Design (RCBD) with three replications per treatment. Collected data were statistically analyzed using MSTAT software (version 4.0, 1985) and mean separation was performed according to the Least Significant Difference (LSD) method, $P \leq 0.05$.

Results and discussion

Total, marketable, non marketable yield and plant productivity: Results indicated that there is no significant differences for total and marketable yields between tuff and soil cultures while yield in culture decreased significantly in the 1st season. In 2nd season, significant differences were observed among the substrates; yield in soil was the highest followed by tuff and sand (Table 1).

For non-marketable yield, no significant differences were observed among different substrates in 1st season, while in the 2nd season yield was significantly higher in soil than tuff and sand cultures (Table 1).

For total yield, no significant difference was observed between tuff and soil, but yield decreased significantly in sand in 1st season. However, in the 2nd season, significant differences among different media were observed (Table 1).

Physical fruit properties: In 1st season, soil gave significantly higher average fruit weight than tuff and sand, but no significant difference was observed among different media in the 2nd season (Table 2).

In addition, no significant difference was observed among different media in 1st and 2nd seasons in respect to fruit length. For fruit diameter, no significant differences among different media were observed in 1st season. However, in the 2nd season it was significantly higher in soil than tuff and sand (Table 2).

Also, in the 1st season tuff gave significantly higher fruit skin thickness than soil and sand, but, no significant differences among different media were observed in the 2nd season (Table 2).

Mineral composition of vegetative growth: Results indicated that in 1st season, nitrogen % was significantly lower in tuff than soil and sand. But, no significant differences were observed among different media in 2nd season. For phosphorous %, no significant differences were observed among different media in 1st season, however, in 2nd season, P% was significantly higher in sand than tuff which was also higher than in soil but the difference was not significant (Table 3). Potassium and dry matter percentage showed no significant differences among different media in 1st and 2nd season.

Percentage of applied water: Calculation for percentage of water applied to tuff and sand was based on the total amount of water applied to the soil. Comparison between tuff and sand with soil show that amount of applied water to tuff culture was 31.8% for 1st season and 33.7% for 2nd season, while for sand, 26.7% for 1st season and 23.7% for 2nd season (Fig. 1).

Tuff, sand, soil, and air temperatures: Results revealed that the lowest difference between the maximum and the minimum temperatures was observed in tuff (Fig. 2). Temperature stress and variation in day and night temperatures may affect several aspects of plant growth, fruit quality and yield in soil as well as soilless

Table 1. Total, marketable, non-marketable yield and productivity of Pepper cv. Reehan

Treatment	Total yield (ton/1000 m²)		Market yield (ton/1000 m²)		Non-market yield (ton/1000 m²)		Yield/plant (kg)	
	2001	2002	2001	2002	2001	2002	2001	2002
Tuff	5.5 a	6.8 b	5.0 a	6.5 b	0.5 a	0.3 b	1.38 a	1.58 b
Sand	2.8 b	2.9 c	2.5 b	2.8 c	0.3 a	0.1 b	0.58 b	0.63 c
Soil	6.0 a	8.7 a	5.7 a	8.1 a	0.3 a	0.6 a	1.30 a	2.00 a

* Mean separation within columns by LSD test, values that don't share the same letter are significantly different at the 5 % level.

Table 2. Average fruit weight, length, diameter and skin thickness of pepper cv. Reehan

Treatment	Fruit weight (g)		Fruit length (mm)		Fruit diameter (mm)		Skin thickness (mm)	
	2001	2002	2001	2002	2001	2002	2001	2002
Tuff	177.0 b	131.7 a	125.3 a	120.3 a	85.9 a	70.3 b	6.3 a	4.7 a
Sand	169.4 b	134.3 a	137.1 a	120.1 a	80.6 a	70.6 b	5.8 b	4.7 a
Soil	200.6 a	150.1 a	134.2 a	131.8 a	84.5 a	74.4 a	5.8 b	4.8 a

* Mean separation within columns by LSD test, values that don't share the same letter are significantly different at the 5 % level.

Table 3. Percentage of nitrogen, phosphorus, potassium, and dry matter of pepper vegetative growth

Treatment	N (%)		P(%)		K(%)		Dry matter (%)	
	2001	2002	2001	2002	2001	2002	2001	2002
Tuff	0.8 b	2.3 a	0.3 a	0.3 b	5.1 a	2.1 a	17.0 a	18.3 a
Sand	1.3 a	2.6 a	0.4 a	0.5 a	4.4 a	2.4 a	17.8 a	19.3 a
Soil	1.6 a	2.6 a	0.3 a	0.4 ab	5.2 a	2.2 a	16.6 a	21.3 a

* Mean separation within columns by LSD test, values that don't share the same letter are significantly different at the 5 % level.

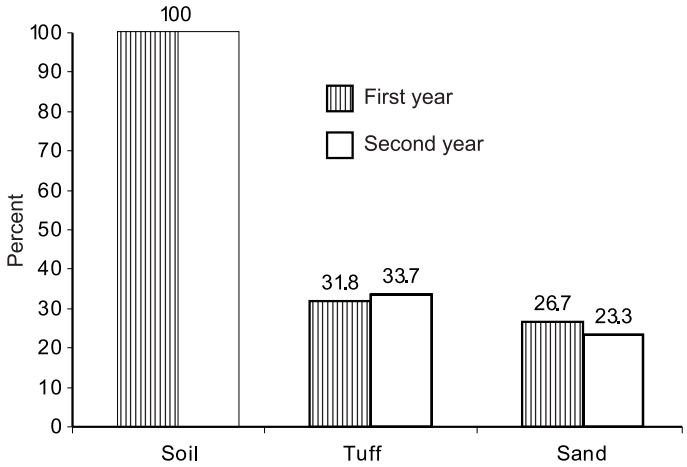

Fig. 1. Percentage of applied water to treatments of pepper in both years

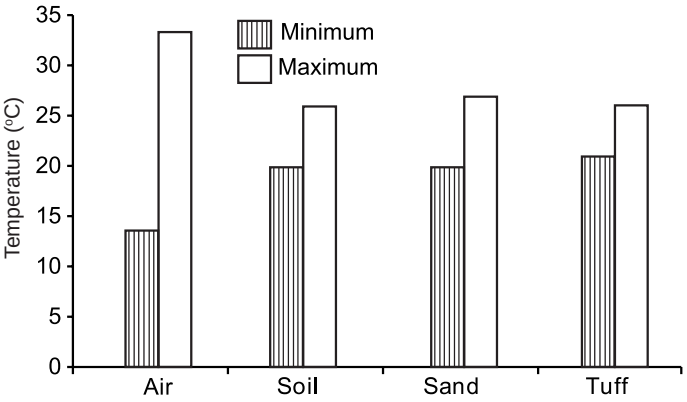

Fig. 2. Temperatures of tuff, sand, soil (at 10 cm deep), and air (at 50 cm height) of pepper.

culture (Hurewitz and Janes, 1983; Kafkafi, 2001; Papadopoulos, 1991; Woitke and Schitzler, 2005). Thus, maintaining optimum root temperature is the main factor in plant production under soilless culture conditions, and the least temperature variation in tuff substrate may reduce the negative effects on yield and quality.

The main disadvantage of using soilless culture systems is the high cost of substrates particularly the imported ones. In order to lower the cost of substrate, locally available material such as black volcanic rock (tuff) may be used. Results of this study indicated that open soilless system using tuff as a substrate may be suitable for sweet pepper production without dramatic changes in its yield or fruit quality. It is concluded that open soilless culture system using tuff substrate may save about 65-70 % of water applied by conventional farmers for sweet pepper under plastic house. However, maintaining the optimum media (root) temperature and controlling the nutrient solution (water) levels may be used as a new technique for growers to manage open system in order to increase oxygen availability to the plant roots.

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Received: April, 2010; Revised: June, 2010; Accepted: August, 2010

Journal of Applied Horticulture, 12(2): 161-164, July-December, 2010

The influence of chlorination on the phytotoxicity and the production of *Zinnia elegans*

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Abstract

Chlorination constitutes a practical and economical chemical control method for the disinfection of recycled nutrient solutions in soil-less growing systems. Although the chlorination can prevent the development of pathogenic organisms, the use of inadequate doses of chlorine could produce damages to the culture and environment. It is necessary to select doses for each plant species that do not cause damages nor produce undesirable effects on the productivity and quality. *Zinnia* sp. in South America has large potential for cultivation as an ornamental potted or vase flower. Tests for disinfection of the recycled nutrient solution were performed with different chlorine quantities (control, 11, 22, 44 and 88 mg L^{-1}) to evaluate the potential phytotoxicity and effects on the flower production (weight and number) of *Zinnia elegans* var. Enana. The production and phytotoxicity were analyzed in relations with the contents of macronutrients (N, P, Ca, and K), sodium and chlorides levels in leaves and related chemical changes (pH, EC and chlorides) in the nutrient solution. The results showed improvement of the development of foliage, roots and the production of flowers with the doses of 11 and 22 ppm, associated to a minor toxicity. The larger doses did not surpass the toxicity levels, although affected the productivity and quality of plants. These results enabled us to select doses under the value of 22 ppm for futures effectiveness test to control pathogens.

Key words: Chlorination, *Zinnia elegans*, phytotoxicity, soil-less culture, disinfection, chemical treatment.

Introduction

From 1980s to 2001, the world-wide area with soil-less cultures production has been increased five times (Rural Industries Research and Development Corporation Program <www.rirdc.gov.au/reports/Ras/01-141.pdf>). The most profitable cultures are tomato, cucumber, pepper, lettuce and cut flowers (roses, gerberas, carnations). Considering Latin American nations, Mexico appears in 17th position with 120 hectares. The future growth of the Closed Soil-less Systems (CSS) in Latin America depends on the development of technology and the adequate assimilation by the production systems, which must be competitive in costs regarding to the technology generated in developed countries.

In many countries, where the soilless culture were developed commercially, the open systems created environmental problems, due to the underground water contamination (Benoit and Cuestermans, 1995; Lopez *et al.*, 1998) caused by leaching of the nutrient solution and resulted into a transition towards the production in closed systems (Tuzel *et al.*, 2000). Some of the advantages of these systems are: diminution of the water consumption (20-30 %), a fertilizer saving (25-45%) and a smaller environmental impact (Magán Cañadas-<www.infoagro.com/abonos/ 917 asp>). However the disadvantages are the demand of a water of superior quality and the risk of a fast dispersion of pathogens through the nutrient solution (pseudofungi such as *Pythium* spp, *Phytophthora* spp, *Plasmopara* spp and *Olpidium* spp), fungi (*Verticillium* spp) or bacteria (*Xanthomonas* spp, *Agrobacterium tumefaciens*, *Erwinia* spp), and virus (Cucumber mosaic virus; Tomato mosaic virus, Lettuce great veins virus and Cucumber mosaic green mottle virus) (George and Biernbaum, 1990; Mac Donald *et al.*, 1994; Stanghellini and Rasmussen, 1994; Van Os *et al.,* 2001). Experiements demonstrated the dispersion of pathogens through the recycled nutrient solution (Magán Cañadas<www.infoagro.com/abonos/docs/9803-3.asp>; George and Biernbaum,1990). The dissemination of diseases is favoured by the homogeneity of variety, the more adequate environmental conditions and the presence of morphologically more adapted waterborne pathogens in the recycled solution (George and Biernbaum, 1990; Mac Donald *et al.*, 1994; Shokes and Mc Carter, 1979; Stanghellini and Rasmussen, 1994).

Chlorination constitutes an alternative chemical control for waterborne pathogens. This method is based on the oxidation capacity of hypochlorous acid. The hypochlorous acid and the ions hypochlorite can prevent the development of pathogenic organisms; their proportion in the water solution depends of the pH and temperature. It is necessary to choose the most effective doses to control the pathogens without causing damages to the cultures productivity and quality. This selection depends on the species, the time of application and the pathogens present in the recycled nutrient solution. The chlorination effect has been studied mainly on pathogens present in cultures of pepper, tomato, cucumber and roses. According to previous studies performed with doses from 0 to 77 ppm of chlorine applied during 12 weeks to some horticultural plants by irrigation, most of the species were not affected by chlorine concentrations under 8 ppm (Frink and Bugbee, 1987). Other studies recommend chlorine doses of 4 to 5 ppm for the control of fungi and bacteria (Escobar *et al.*, 2003; Poncet *et al.*, 2001).

The major risk of the addition of high quantities of chlorine is the phytotoxic effects that may vary according to the sensitivity

of species to chlorine concentration. This phytotoxicity is the result of the oxidation effect of chlorine on the cellular walls and membranes and on the cellular content (plant metabolism) or the sodium ion accumulation in the recycled system. The continuous addition of sodium hypochlorite can lead to sodium ion accumulation and also cause an alteration in the Na/K relation in the nutrient solution. The culture growing media is a complex system and can cause difficulty in the evaluation of chlorine phytotoxicity. The residual chlorine can produce phytotoxicity in cultures grown on inert substrates or with nutrient film techniques (NFT); however in cultures grown on organic substrates the residual chlorine is quickly inactivated.

Zinnia (*Z. elegans* L.) is cultivated during summer when temperature may affect chlorination, susceptible to a great number of pathogens common to other flower species (gerbera, alstroemeria) developed in experimental soilless cultures in Argentina (Chase, 1987; Palacios *et al.*, 1991) and good adapted to irrigation with nutrient solution (fertigation). Preliminary tests with Zinnia determined that 5 and 11 ppm doses elevated the chloride contents in the recycled solutions, although not exhibiting phytotoxicity symptoms, and recommended to test other doses to find out the maximum tolerance to chlorine of this species (Premuzic *et al.*, 2004).

The effects of the addition of different chlorine quantities on phytotoxicity and production (weights and amount of flowers) of *Z. elegans* var. Enana, were studied and related with both content of macronutrients (N, P, Ca, K), sodium and chlorides in plants and the changes in chlorides, pH, EC of the nutrient solution**.**

Materials and methods

A closed soil-less system was developed in a greenhouse of metallic structure and with polyethylene cover with zenithal and lateral ventilation, belonging to the Chair of Floricultura of the FAUBA. The flower pots were kept in the greenhouse and watered with automatically recirculation of drainage. Seeds of *Z. elegans* L. *var.* Enana were germinated and transplanted after 15 days of the emergence to white polyethylene flowerpots of 15 cm Ø and 17 cm height filled with perlite. A plant with its corresponding drip emitter was placed at each flowerpot. A drip irrigation system was installed, applying a volume of 2 L h^{-1} by 6/7 daily irrigations of 4 minutes each one. The pots were placed on wood benches with a 5% slope to allow the flow of leachates to the storage tank at the end of the bench.

Five treatments of sodium hypochlorite including a control (without the addition of sodium hypochlorite) were applied. The chlorine doses of treatments were: (1) control: without hypochlorite; (2) 11 mg L^{-1} or ppm; (3) 22 ppm; (4) 44 ppm and (5) 88 ppm of sodium hypochlorite. The different chlorine doses resulted from a dilution of commercial sodium hypochlorite to 100 ppm (stock solution) and from the corresponding dilutions of the stock solution. Chlorination was done to the spending tank with the nutrient solution. The Hoagland-Arnon solution was used that contributed (ppm): 150 N, 190 K; 60 P; 120 Ca; 40 Mg; S 40; and micronutrients Fe 2; Mn 1.5; B 0.2; Cu 0.07; Zn. 0.07; Mo 0.05; pH was 5.0 and CE 1.5 mS/m^2.

The effects caused by the addition of different chlorine quantities were evaluated on the production (fresh and dry weight), some commercial quality factors (phytotoxicity, number of flowers) and the levels of macronutrients in leaves. All the measured values were confronted with the contents of sodium and chloride in leaves due to the possible toxicity and the chemical changes (pH, electrical conductivity and chloride) in the nutrient solution.

Phytotoxic symptoms were monitored analyzing the relation: number of leaves with necrosis/total number of leaves per plant x 100. The necrosis at the edges of leaves was measured every four days.

A digital balance ACCULAB GS200 was used for the assessment of weight (aerial and root fresh weights, and aerial dry weight on material dried in stove at 70°C for 48 h).

The flowering was quantified as the total number of flowers per plant.

Principal macronutrients levels were determined: N (Kjeldahl), P (colourimetric), Ca (atomic absorption), K and Na (flame photometry). Chlorides were quantified by a volumetric precipitation.

The characterized chemical changes of the nutrient solution were electrical conductivity (conductimeter), pH (potentiometer) and chloride concentration (volumetric precipitation). The consumed volume of the nutrient solution and the corresponding doses of chlorine and fertilizer were refilled.

The culture was developed between October and February, starting the chlorination of the nutrient solution one week after the transplant of plants from growing media to the pots with perlite.

A totally randomized experimental design with the pots as experimental units was carried out with 15 replicates per treatment. The results were statistically analyzed by comparison of means (ANOVA) and in case of significant differences ($P \leq 0.05$) post doc test (LSD) using the program SPSS (Field, 2000).

Results and discussion

Production: The 44 and 88 ppm treatments affected the flower production and the aerial fresh weight, producing a decrease of 40-70% in the aerial biomass and 50 % in the production of flowers. The treatments with 11 and 22 ppm resulted with the larger values of aerial fresh weight. The root fresh weight decreased with increasing quantities of chlorine, and with a similar trend as in aerial weight, the 44 ppm and 88 ppm treatments presented the minor values. A highly significant positive correlation (P=0.01) among the fresh aerial and root weight and the number of flowers was observed. Percent aerial dry matter increased with the 44 and 88 ppm treatments and 88 ppm showed significant differences with 11 and 22 ppm treatments. The values indicate the presence of larger dry matter % with the larger dose of chlorine (Table 1).

A different behaviour was observed in the macronutrients contents. The contents of P (0.14-0.20 %) and Ca (1.3-1.71%) did not present significant differences among treatments.

The contents of N (2.8- 4.29 %) and K (3.65-5.6 %) presented the larger values with the control and 11 ppm treatments. The high N contents with the 88 ppm treatment were a consequence of a concentration effect due to the minor aerial biomass associated to

Table 1. Vegetative growth and flowering in *Z. elegans* under different chlorine treatments

Treatments chlorine (ppm)	Aerial fresh weight (g)	Aerial dry matter (%)	Roots fresh weight (g)	Number of flowers
Control	74.1a	22.3ab	9.7bc	1.6b
11 ppm	85.9a	18.4b	15.2ab	2.14a
22 ppm	101.4a	17.5b	17.6a	2.57a
44 ppm	46.8b	25.4ab	8.9bc	1.57b
88 ppm	32.3b	30.2a	4.9c	1.43b

Values are average of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

Table 2. P, Ca, N and K content in leaves of *Z. elegans* under different chlorine treatments

Treatments chlorine (ppm)	Ca (%)	P (%)	N (%)	K (%)
Control	1.3a	0.16a	4.3a	5.3a
11 ppm	1.4a	0.15a	3.5a	5.6a
22ppm	1.6a	0.15a	2.9b	4.1b
44 ppm	1.7a	0.14a	2.9b	3.6b
88 ppm	1.3a	0.20a	3.8a	4.1b

Values are average of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

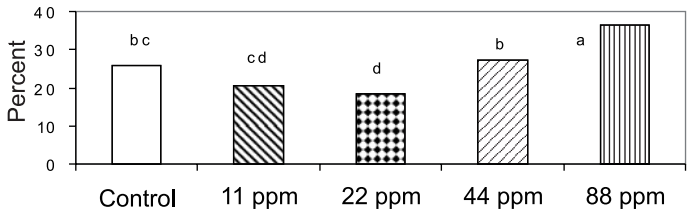

Fig. 1. Phytotoxicity in leaves of *Z. elegans*. Bars represent the average values of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

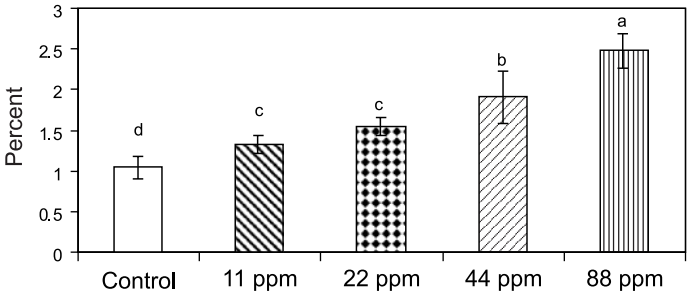

Fig. 2. Contents of chlorides (%) in leaves of *Z. elegans*. Bars represent the average values of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

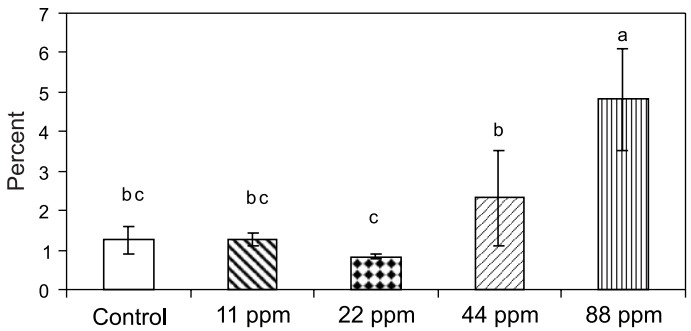

Fig. 3. Sodium (%) in leaves of *Z. elegans*. Bars represent the average values of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

the 88 ppm treatment. All values of N, Ca and K were adequate, while the values for P were less than the required for ornamental species (Reed, 1999; Wolf, 1996) (Table 2).

Phytotoxicity: The 88 ppm treatment presented a significantly higher percentage of necrotic leave's tissue as compared to the rest of the treatments while the 22 ppm treatment presented a low percentage of damage, although with no significant differences with the 11 ppm treatment, the last one and the control showed an intermediate damage (Fig. 1).

The damage and necrosis of leaves exhibited a positive correlation with the chloride and sodium content. The 88 ppm treatment induced more than 40 % damage, while with the 11 ppm and 22 ppm treatments the proportion of necrotic leaves was significantly less than the other treatments. The phytotoxicity symptoms caused by chlorine developed first at the tips of the leaves and soon as the toxicity progressed moved throughout the edges. The chloride and sodium contents in leaves increased significantly with the rise of the quantities of applied chlorine. The sodium contents showed differences among treatments and 88 ppm treatment recorded higher values for both sodium and chlorides (Fig. 2 and 3).

The values for chlorides in leaves were between 1.04 and 2.48 %, the 44 ppm and 88 ppm treatments presented contents 100 and 200% larger than the 22 ppm treatment, respectively. There was a negative correlation between chloride contents in the leaves and the fresh weight of plant. The levels of sodium in leaves were between 0.83-4.81%, increasing significantly with the increase in the chlorination dose. The 44 ppm and 88 ppm treatments showed the sodium levels that surpassed the normal levels in vegetative tissue (Chapman and Pratt, 1979).

The chemical properties measured in the recycled solution (Table 3) did not present significant differences among treatments for the electrical conductivity (2.45- 2.7 ds/m). The pH (6.3-6.7) increased significantly with the chlorination, although values were not critical for the culture. The chloride content showed significant differences with two clearly separated treatment groups: in one group control, 11 and 22 ppm treatments were included and another group had 44 and 88 ppm treatment. Although, none of the treatments surpassed the 144 limit of toxicity (Reed, 1999). The addition of chlorine did not affect the values of pH or EC above the ranks recommended for ornamentals plants (Chapman and Pratt, 1979).

Although the doses of 44 and 88 pm did not surpass the toxicity limits they affected the productivity parameters. The 22 ppm and 11 ppm doses favoured the best development of foliage, roots and production of flowers and caused the minor toxicity. The properties measured in the recycled nutrient solution did

Table 3. Chemical properties measured in the recycled solution

Treatments chlorine (ppm)	CE (mS/ m^2)	pH	Chloride (ppm)
Control	2.70a	6.31b	85.9b
11 ppm	2.45a	6.59a	85.2b
22 ppm	2.45a	6.51a	89.1b
44 ppm	2.53a	6.56a	115.0a
88 ppm	2.52a	6.70a	141.0a

Values are average of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

not present critical values for the species. All the contents of macronutrients were adequate for the species. The results suggest not to use chlorine doses higher than 22 ppm for the disinfection of *Z. elegans* culture in closed soilless systems.

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Received: August, 2010; Revised: October, 2010; Accepted: November, 2010