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Control of Powdery Mildew in Cut Roses using Natural Products in the Greenhouse

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ABSTRACT

Roses grown under greenhouse cultivation are commonly affected by diseases and regular fungicide applications are commonly used. The aim of this research was to identify natural products that can substitute and reduce the health and environmental impacts of typical chemical treatments in the control of powdery mildew [(*Podosphaera pannosa* (Wallr. Fr.) de Bary] and grey mould (*Botrytis cinerea* Pers.). Treatments were applied in the greenhouse on the cut rose cultivars Sunluck (Gold Strike®) and Nirpbredy (New Fashion®) growing in a soilless system. Oregano and clove essential oil at 0.5 ml/l (an emulsifier was added) and sodium bicarbonate ($NaHCO_3$) at 4 g/l were sprayed on rose plants to runoff. The controls were untreated roses and a standard chemical control (the fungicides Dinocap, Triadimenol, Dimethomorph+Sulphur, Bupirimate, Dithianon, Iprodione and Thiram) was applied in rotation. Sprays were applied every 7-10 days after the first symptoms of disease appeared. Disease incidence was checked on 100 leaflets and symptoms were evaluated using a scale from 0 (no disease) to 7 (76-100% infection). At three different times (June and November 2005 and January 2006) biometric data (stem height, stem diameter, stem flexibility, flower diameter, number of petals/flower and thorniness) and colorimetric analysis of the leaves were evaluated in order to determine the effect of treatments on cut rose quality. Treatment with essential oils and $NaHCO_3$ was able to control the incidence and the severity of powdery mildew on roses. Ambient infection of grey mould was low and comparisons between treatments were not possible. Significant differences among the chemical and natural product treatments were found for all traits examined. Although $NaHCO_3$ treatment controlled powdery mildew to a greater extent than essential oils, it resulted in slight phytotoxicity. These results indicate the potential use of natural products to control powdery mildew of roses and could be a good alternative to chemical fungicides. However, differences in response to powdery mildew depend on the cultivar susceptibility, period of treatments and level of control needed.

Keywords: crop protection, grey mould, natural products, powdery mildew, quality control, *Rosa hybrida*
Abbreviations: EO, essential oil; **$NaHCO_3$**, sodium bicarbonate; **RH**, relative humidity

INTRODUCTION

Roses are among the most important cut flowers grown in greenhouses. High humidity and other conditions in the greenhouse can increase the incidence of disease and represents a constant threat to the cultivation of cut roses. The control of rose diseases can be difficult and relies on reducing inoculum, limiting pathogen spread, making the environment less conducive to disease, excluding the pathogen, exploiting intrinsic plant resistance and enhancing biological control (Jarvis 1992). Many diseases affect roses and are caused by fungi, bacteria and viruses. Powdery mildew and grey mould are probably the most widely distributed and dangerous fungal pathogens for cut rose production in the greenhouse environment. Powdery mildew in rose, caused by *Podosphaera pannosa* (Wallr. Fr.) de Bary, appears on epigeous parts of the plant, especially on young tissue. The optimal conditions for powdery mildew are temperatures of 21°C for germination of conidia and 18-25°C for mycelial growth and a high relative humidity (RH) (97-99%) (Horst 1986). Grey mould, caused by *Botrytis cinerea* Pers., mainly occurs in storage of harvested cut roses. Infected rose buds may fail to open and drop and petals become brown (especially the tip), soft and covered with a greyish brown mycelial growth. The development of grey mould is favoured by a temperature of 15°C, high RH and persistent tissue wetness (Horst 1986). The control of both diseases is mainly achieved by rotation of chemical products because the continuous use of a single fungicide may lead to the selection of pesticide-resistant pathogen populations and a general loss in efficacy. Because of continuous fungicide applications, many of which are characterized by a high toxicological environmental and human impact, natural products such as essential oils (EOs), water extracts, bicarbonates, etc. could offer an alternative system of disease control. The antimicrobial activities of EOs obtained from aromatic and medicinal plants have been known for a long time. The first research dates back to 1921 (Macht *et al.*), and since then a wide range of natural substances extracted from plants such as oregano, clove, thyme, sage, coriander, lavender, geranium, basil, neem and many others were tested against a large number of plant and human pathogens (Morris *et al.* 1979; Northover *et al.* 1993; Daayf *et al.* 1995; Wilson *et al.* 1997; Bhaskara Reddy *et al.* 1998; Awuah *et al.* 2001; Marino *et al.* 2001; Scarito *et al.* 2002). In addition, the antioxidant activity of these substances was recently evaluated (Baratta *et al.* 1998; Economou *et al.* 1991).

In regards to the antifungal activity of bicarbonates, it has been recently verified experimentally (Lahoz *et al.* 2000). Sodium and potassium bicarbonates showed a positive effect on rose plants against *P. pannosa* and black spot caused by *Diplocarpon rosae* Wolf (Horst *et al.* 1992; Salamone *et al*. 2007) and on sweet red pepper against *Leveillula taurica* (Lév.) (Fallik *et al.* 1997).

The aim of the present study was to: a) determine the

Received: 7 August, 2008. Accepted: 7 May, 2009.

Original Research Paper

effectiveness of oregano and clove EOs and sodium bicarbonate ($NaHCO_3$) in controlling natural infections of powdery mildew and grey mould of rose; and b) compare the efficacy of traditional chemical treatments and natural products on rose quality.

MATERIALS AND METHODS

Greenhouse environment and plant materials

The trial was conducted at *C.R.A.– Unità di Ricerca per il recupero e la valorizzazione delle Specie Floricole Mediterranee (CRA-SFM)* in Bagheria (near Palermo, NW Sicily, 38°5' N, -13°30' E, 23 m above sea level), in an unheated East-West oriented greenhouse (34 x 16 m) with a metal structure and polyethylene cover (Ø 0.15 mm). Rose plants of *Rosa hybrida* L. 'Sunluck' (Gold Strike®) and 'Nirpbredy' (New Fashion®), grafted on 'Fun Jwan Lo' (=*Rosa indica major*) rootstock, were grown in 80L white plastic bags filled with a perlite and coconut coir dust mixture (1:1, v/v), in an open-loop system. 'Sunluck' has bright yellow petals, large flower heads, medium/long, thin stems and is fragrant (introduced in 1999 by Franko Roses, New Zealand). 'Nirpbredy' is a bicolor rose with red on the inner (adaxial) petal surface and cream on the reverse. It has large flowers and is favoured for use in bouquets (introduced in 2000 by NIRP International, France).

Planting was accomplished in January 2003. Each bag (100 × 50 cm) supported 10 plants (2 rows of 5 plants) with a density of 4.5 plants/m^2 of cultivated soil. Bags were placed on polystyrene blocks in order to raise them from the ground, with a slight slope (0.5%) to allow free drainage of excess nutrient solution. Water as well as macro- and micronutrients were supplied to the plants through a drip fertigation system (1 dripper/plant, 2 L/h) controlled by a timer. The nutrient solution had the following composition: 140 N-NO_3; 40 N-NH_4; 50 P; 200 K; 120 Ca; 30 Mg; 1.3 Fe; 0.2 Cu; 0.2 Zn; 0.3 Mn; 0.2 B and 0.03 Mo mg/L (see **Appendix 1** and **2**). Daily irrigation timing was based on substrate matric potential by using electronic low-tension tensiometers. In addition, a nebulization system was used to ensure a constant RH value (about 60%) and black plastic shade cloth (50% light reduction) was placed above the plants during the warmest months (June-September).

Disease control treatments

Natural products used to control rose diseases were oregano and clove EOs (Titolchimica, Pontecchio Pol. Rovigo, Italy) at 0.5 ml/L and $NaHCO_3$ at 4 g/L. A randomized complete block design with 4 replications per treatment was adopted. Each replication consisted of 20 plants distributed in two bags (each bag containing 10 plants of the same cultivar). In order to reduce surface tension and allow a uniform wetting of plants, the EOs were emulsified in a commercial product (Fitoil®, Agribiotec srl, Italy) based on soy (regulation CEE 2092/91). Natural product treatments were compared with standard chemical products used in rotation (one product to control powdery mildew + one for grey mould) to prevent resistance from developing to control powdery mildew [Dinocap (25 ml/hl), Triadimenol (300 g/hl), Dimethomorph+Sulphur (75 ml/hl+250 g/hl) or Bupirimate (250 ml/hl)] and grey mould [Dithianon (10 g/hl), Iprodione (300 ml/hl) or Thiram (20 g/hl)]. Additionally, treatments of Tebufenpyrad+Hexythiazox (60 g/hl+40 g/hl), Acrinathrin+Hexythiazox (80 g/hl+20 ml/hl), Abamectin (70 ml/hl) and Pymetrozine (100 g/hl) were also applied, when needed, to control red spider mites (*Tetranychus urticae* Koch.) and greenhouse white fly (*Trialeurodes vaporariorum* Westw.).

All sprays were applied weekly after the appearance of the first symptoms of powdery mildew and grey mould infection. Plants were treated from January to December 2005 using a compression sprayer. Disease severity of powdery mildew was checked 24 hours before and one week after each treatment on 20 complete 5-leaflet leaves per replication (100 leaflets/replication) randomly chosen from the new shoots and the incidence was evaluated on individual leaflets using the following scale of symptoms adopted from Pasini *et al.* (1997): 0 = no disease observed; 1 = up to 2.5% of the leaf blade covered by powdery mildew; 2 = 2.6-5%; 3 = 6-10%; 4 = 11-25%; 5 = 26-50%; 6 = 51-75%; and 7 = 76-100%. Incidence was calculated with the formula used by McKinney (1923). Roses were harvested by cutting to the second 5-leaflet leaf from stem origin and grey mould incidence was evaluated during flower harvest by recording the percentage of stems with infected buds or petals.

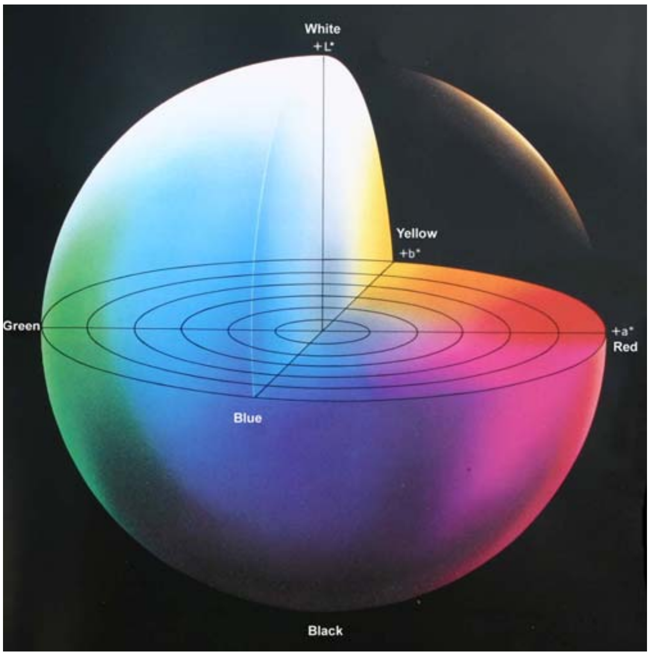

Fig. 1 CIELab colorimetric diagram. Picture elaborated from: La comunicazione precisa del colore. Il controllo del colore: dalla sensazione alla strumentazione (Minolta 1994). With kind permissions from Minolta.

In order to study the effect of treatments by natural products on the quality of rose production, the following biometric data were evaluated on a sample of 20 representative flower stems randomly collected per treatment: stem height (cm) measured from the base of the flower bud to cut point; flower stem basal thickness; stem flexibility (expressed in sexagesimal degree) calculated as the measurement of the rose bud flexion with respect to the horizontal plane; thorniness evaluated by using an empirical scale expressed in different classes (from 0 = <5% thorniness to 5 = >80%) according to the amount of prickle present on the rose stem. A colorimetric analysis of the adaxial side of 20 leaflets was also carried out for each treatment randomly collecting the first 5 fully expanded leaves from the tip of the stem using a tri-v *stimulus* colour difference meter (Minolta CR300; Minolta, Tokyo, Japan) to determine L* and colorimetric coordinates a* and b*; $L^* a^* b^*$ or CIELab (**Fig. 1** elaborated from: "Il controllo del colore: dalla sensazione alla strumentazione", Minolta 1994). Colour space is an international standard for colour measurements, adopted by the *Commission Internationale d'Eclairage* (CIE) in 1976. L^* is the luminance or lightness component which ranges from 0 to 100 and parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components which range from −120 to 120 (Segnini 1999; Papadakis 2000; Yam *et al.* 2004).

Data were statistically analysed by analysis of variance using SPSS 9.0 for Windows (Chicago, Ill) and Duncan's multiple range test was used for mean separation at $P \leq 0.05$. Percentage data were arcsine transformed prior to analysis.

RESULTS

Diseases present

Data reported in both figures and tables only refers to infections caused by *P. pannosa*. The presence of grey mould was sporadic throughout the trial period (an overall incidence of infection of about 2-3%) and is not presented.

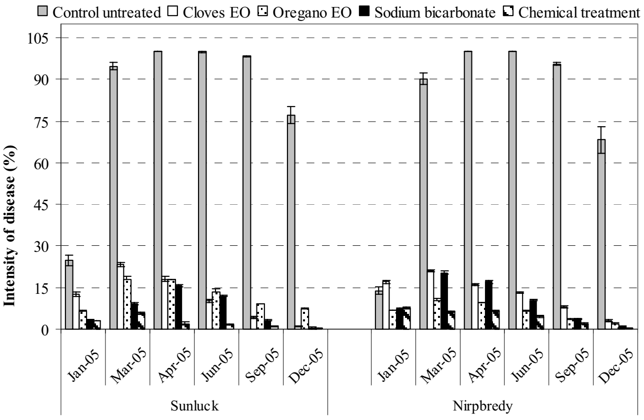

Fig. 2 Average disease severity (%) of powdery mildew of 'Sunluck' and 'Nirpbredy' roses treated with clove EO, oregano EO and $NaHCO_3$ in comparison with chemical treatments and an untreated control (values are means ± SE).

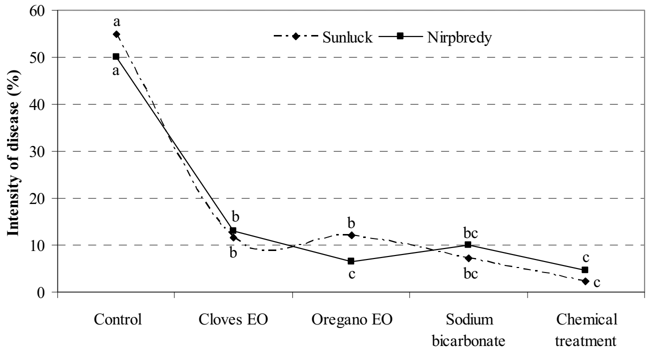

Fig. 3 Average disease severity (%) of powdery mildew of rose treated with natural products in comparison with untreated rose cultivars leaves ('Sunluck' and 'Nirpbredy').

Powdery mildew control

Fig. 2 shows the disease severity observed over the study on rose leaves according to treatments (natural substances in comparison with traditional chemical products). On 'Sunluck', clove and oregano EO treatments constantly inhibited powdery mildew compared to the untreated control. The single highest disease incidence values for clove and oregano EOs was observed in March with infections of 23.5 and 18.1%, respectively. Treatments by EOs were more effective from April to December reducing the average infection on leaves across these months to 7.4% with oregano and 1% with clove EOs and was far lower than that of the untreated control (77.2 %). Sodium bicarbonate had lower disease incidence than treatments with oregano and cloves, and it controlled the disease throughout the period of the trial, except in April (15.7%), reaching the lowest percentage of infection in December (0.4%). In December, control with $NaHCO_3$ was as good as with traditional chemical products.

On 'Nirpbredy' the oregano treatment showed more constant control throughout the duration of the trial than clove oil with a slight peak value of infection in March (10.4%). Sodium bicarbonate was not as effective as oregano EO even when, in December, the disease severity never exceeded 1%, showing an effectiveness comparable with that of the traditional chemical treatment. In regard to the clove EO treatment, there was less efficacy than the other treatments and this was generally different than for 'Sunluck' (**Fig. 2**). For both cultivars, however, traditional chemical treatments always controlled the disease more effectively than the natural products.

On both cultivars the natural products were effective in reducing infections of powdery mildew (**Fig. 3**), always displaying vastly reduced disease intensity relative to the untreated control. However, oregano EO significantly reduced the infection on 'Nirpbredy' compared with clove EO, while $NaHCO_3$ was more effective on 'Sunluck'.

Effect of treatments on the quality of cut roses

'Sunluck' showed a significant increase in stem height and thorniness when treated with oregano EOs relative to bicarbonate and for stem height when oregano EO is compared to the traditional chemical treatment (**Table 1**). In 'Nirpbredy' the cloves EO treatment had the longest stems (47.8 cm), the oregano (42.9 cm) and traditional chemical treatment (47.5 cm) were comparable, and the $NaHCO_3$ treatment had the shortest stems (38.3 cm). Stem flexibility varied in 'Sunluck' with $NaHCO_3$ showing greater flexibility (3.5°) than all other treatments. The traditional chemical treatment showed the highest flexibility (4.7°) for 'Nirpbredy'.

The different treatments affected luminance and colorimetric coordinates on both cultivars. Significantly higher luminance was recorded on traditional chemically-treated leaves relative to natural products on both cultivars (**Table 2**). The lowest luminance in both cultivars was consistently observed on $NaHCO_3$ treated leaves. There was a 5.9 point difference on average between the $NaHCO_3$ and traditional chemically-treated leaves. Oregano and clove EO-treated plants showed intermediate leaf luminance in both cultivars.

In 'Sunluck', a* colorimetric coordinate was the most distant from 0 (toward the negative direction) in the $NaHCO_3$ treatment (i.e. more intense green colour). The traditional chemical treatment showed the least intense green colour, significantly different than the other treatments. Values of a* that were considered intermediate or even good were recorded on oregano and clove EO-treated roses (**Table 2**). The same trend was observed in 'Nirpbredy' with lower differences between the four treatments. Oregano and clove EO-treated leaves showed a more negative b* coordinate value than traditional chemical and $NaHCO_3$ treated plants (i.e. higher blue colour saturation) for 'Sunluck'. However, for 'Nirpbredy' $NaHCO_3$ and oregano EO had significantly more negative b* values (**Table 2**).

Table 1 Effect of treatment with natural products on key biometric parameters compared to standard chemical products.

Cultivar	Treatments	Plant height (cm)	Stem flexibility[(a)]	Stem diameter (mm)	Thorniness[(b)]
Sunluck	Sodium bicarbonate	45.9 c[(c)]	3.5 a	4.1 b	2.0 c
	Oregano EO	52.1 a	2.6 b	4.9 a	2.8 ab
	Cloves EO	50.2 ab	2.6 b	4.4 ab	2.9 a
	Chemical products	47.5 bc	2.5 b	3.9 b	2.5 b
Nirpbredy	Sodium bicarbonate	38.3 c	3.8 b	4.5 b	2.6 b
	Oregano EO	42.9 b	3.0 c	4.7 b	3.1 a
	Cloves EO	47.8 a	3.1 bc	5.2 a	3.1 a
	Chemical products	43.1 b	4.7 a	4.8 ab	2.7 ab

[(a)] expressed in sexagesimal degree.
[(b)] expressed in classes from 0 (<5% thorns) to 5 (>80% thorns).
[(c)] Means followed by with the same letter do not differ significantly using Duncan's multiple range test at $P \leq 0.05$.

Table 2 Colour of leaves as affected by the different treatments.

Cultivar	Treatments	L*	a*	b*
Sunluck	Sodium bicarbonate	42.4 c	-6.2 c	-1.2 a
	Oregano EO	46.5 b	-4.7 b	-1.7 b
	Cloves EO	46.7 b	-4.4 ab	-1.6 b
	Chemical products	49.0 a	-3.9 a	-0.9 a
Nirpbredy	Sodium bicarbonate	42.7 d	-4.9 b	-2.2 b
	Oregano EO	45.5 bc	-4.4 a	-1.7 b
	Cloves EO	46.4 b	-4.6 ab	-0.9 a
	Chemical products	48.1 a	-4.4 a	-0.8 a

L = Luminance; a and b chromaticity coordinates (+ a = direction of red, - a = direction of green, + b = direction of yellow, - b = direction of blue)
[c] Means followed by with the same letter do not differ significantly using Duncan's multiple range test at $P \leq 0.05$.

DISCUSSION

Clove and oregano EO and $NaHCO_3$ treatments were able to consistently control the the disease intensity of *P. pannosa* on roses in comparison to the untreated control. Sodium bicarbonate treatments were comparable to the traditional chemical products. Between the EO treatments, oregano EO showed better control of infection than clove EO. Cultivars susceptibility to powdery mildew and sensitivity to the treatments must also be considered. Both EOs did not show apparent phytotoxicity symptoms, however, a reduction in stem length was observed with the $NaHCO_3$ treatment, suggesting a slight phytotoxic effect. The results obtained in this study seem to confirm the antimicrobial activity of EOs. Antimicrobial activity has been attributed to the complex chemical composition of EOs characterized by monoterpenic phenols, especially thymol, carvacrol, eugenol (constituents of oregano and clove EOs) and aromatic compounds derived from phenylpropane (Battistutta *et al.* 1995; Bruneton 1995). However, the most attractive aspect of using EOs as crop protectants is their low mammalian toxicity; some EOs (thymus, oregano, cloves, anise, basil, cumin, etc.) have relatively high acute oral LD_{50} value of 2-3 g kg^{-1} in rat (Isman 2000).

Regarding colorimetric analysis, favourable results have been obtained by natural treatments; the colour of the leaves appeared healthy with an attractive green colour compared to traditional chemically-treated leaves. The visual appearance of floral products is the first parameter of quality evaluated by consumers. In the marketplace the colour of petals and leaves are very important for consumer acceptance. Plants in the greenhouse are subject to many treatment applications that can affect the hue of plant tissues and therefore the economic value. The employment of a new product should be preceded by the study of its effect on colour. The determination of colour can be carried out by visual (human) inspection or by using a colour measuring instrument. Although human inspection is quite robust, even in the presence of changes in illumination, the determination of colour can be subjective and variable from observer to observer. In order to carry out a more objective colour analysis, colour standards are valuable tools providing a reference. Unfortunately, their use implies a slower rate of inspection and requires specialized training of the observers. For these reasons it is recommended to determine colour through the use of colour measuring instrumentation (León *et al.* 2006).

ACKNOWLEDGEMENTS

The authors express their thank to Dr. G.V. Zizzo and Dr. S. Agnello of *Unità di Ricerca per il recupero e la valorizzazione delle Specie Floricole Mediterranee (CRA-SFM)* and Prof. A. Sciortino of *Dip. Agronomia Ambientale e Territoriale (DAAT) – Università degli Studi di Palermo* (Italy) for their kind collaboration.

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Appendix 1 Salts typology and used amounts for the preparation of the nutrient solution.

Salt	g/L
Iron-EDTA	0.04
Ammonium nitrate	0.15
Potassium nitrate	0.18
Calcium nitrate	0.4
Magnesium nitrate	0.15
Potassium monophosphate	0.5
Magnesium sulphate	0.6

Appendix 2 Total amount (**mg/L**) and recipe (single element amount and percentage) of micronutrients mid used for the preparation of the nutrient solution.

Micronutrients (commercial prepared mid)	41.0
Zn	2.0 (5%)
Mn	1.5 (4%)
Cu	0.4 (1%)
B	0.1 (0.4%)
Mo	0.04 (0.1%)