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Effects of Light, Temperature, and 2′,4′-Dichloro-1-Cyanoethanesulphonanilide on Degreening of Regreened 'Valencia' Oranges[1]

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Abstract. Degreening occurred when 1000 ppm 2′,4′-dichloro-1-cyanoethanesulphonanilide (R33417) was applied preharvest or postharvest to regreened 'Valencia' oranges [*Citrus sinensis* (L.) Osbeck] when light was present. Part of the light effect may have been due to increased rind temperatures because some degreening occurred when treated fruit were held in darkness at 40°C. Little or no degreening, however, occurred in darkness at 25 or 4°C. Some postharvest degreening occurred in the absence of R33417 in darkness at 40°C and considerable degreening occurred on fruit which were exposed to light but not treated with R33417 even when rind temperatures were considerably lower than 40°C. Results obtained with untreated fruit agree with the currently accepted view that intense light can cause chlorophyll destruction, however, excised orange fruit were much more susceptible to degreening by intense light than attached citrus leaves. Based on the combined effects of light, temperature, and R33417 on degreening we speculate that the chemical potentiates the destructive effects of intense light and high temperatures on chlorophyll. Evidence that light does not convert R33417 into a biologically active compound, and preliminary evidence that R33417 reduces the extent of ethylene degreening are presented.

During the maturation of 'Valencia' oranges, the chlorophyll content of the rind decreases and carotenoids accumulate. In production areas where maturation coincides with warm days and cool nights, virtually all of the chlorophyll disappears. If mature fruit are kept on the tree during the summer months, which is a common practice in many parts of the world, chlorophyll returns to the rind and carotenoid content decreases. This is referred to as regreening. The magnitude of regreening depends, among other things, on environmental conditions and cultural practices, but these aspects of the problem are beyond the scope of this paper.

Degreening of regreened fruit can be accomplished by prolonged exposure of harvested fruit to ethylene in a warm, humid atmosphere. However, this procedure hastens rind senescence (1) and thus increases storage and shelf-life problems. In view of the importance of regreening to the fresh fruit market, we are interested in alternative methods to combat the problem. During a search for compounds which will modify the pigmentation of citrus fruits, we confirmed that R33417 (Fig. 1) can cause degreening, as originally suggested by ICI Plant Protection Limited. Our purpose was to document the biological activity of R33417 and to describe the influence of light and temperature on degreening and on the effectiveness of the compound.

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Materials and Methods

Preharvest and postharvest treatments were applied to selected regreened fruit by immersion for 3 min in 95% ethanol or in 95% ethanol containing 1000 ppm R33417. Chlorophyll content of the rind was measured at 9 specific spots on each fruit at the beginning of the experiment and at specified times thereafter with a reflectance meter developed by Wallihan (4) which reads directly in absorbance units. Based on our calibration data, a decrease in absorbance of 0.10 represents a loss of 4.6 μg chlorophyll cm^{-2}. The relation between absorbance over the range of 0.20 to 0.40 and chlorophyll from 0.6 to 9.8 μg cm^{-2} was linear. To put this into perspective, a fruit with an absorbance of 0.20 appears to be essentially free of chlorophyll while a fruit with an absorbance of 0.40 is definitely green. A randomized complete block design was used with blocking based on initial chlorophyll content of the rind. The average absorbance change (ΔA) per fruit was used in statistical evaluations. Light, temperature, and humidity conditions for the postharvest experiments were controlled.

The possibility that light converts R33417 into a biologically active

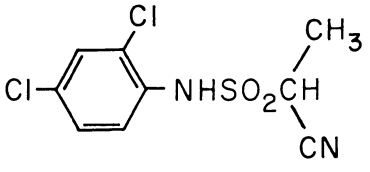

Fig. 1. Structural formula of 2′-4′-Dichloro-1-cyanoethanesulphonanilide (R33417).

compound was examined in a preliminary way. For this study the compound was exposed to UV light and degreening activity of the irradiated material was determined in a fashion similar to the procedure described above. Ultraviolet light was selected since the absorption spectrum of an ethanol solution of the compound lies totally within the UV region.

Results and Discussion

Preliminary research with R33417 on regreened 'Valencia' oranges showed that preharvest applications caused degreening on the tree and that postharvest treatments, in which fruit were kept in dark storage at 25°C, caused no appreciable loss of chlorophyll. These contrasting results suggested that R33417 may only act as a degreening agent when light is present. Preharvest experiments confirmed the apparent importance of light to the chemically-induced degreening reaction (Table 1). There are 3 obvious ways in which light may be involved: it may influence one or more biological reactions of the tissue through photochemical effects; it may convert R33417 into a biologically active compound, which, when applied to the tissue requires no light; or intense radiation may influence the system through raising rind temperatures.

When R33417 was subjected to UV radiation, qualitative changes were seen in the absorption spectrum of the material but no qualitative changes were seen in degreening ability. In postharvest degreening studies, neither R33417 nor the irradiated material caused degreening when treated fruit were kept in darkness at 25°C but both solutions induced degreening when fruit were exposed to the visible portion of the spectrum. However, the UV-irradiated sample caused less degreening than R33417.

In all of the postharvest studies, essentially no UV light reached the fruit because a 6 mm glass plate separated the fruit from the lamps. The fact that degreening occurred provides further support for the conclusion that light does not exert its effect by converting R33417 into a biologically active compound.

In a preharvest experiment more degreening was caused by R33417 with exposed fruit than with those inside the tree canopy, and no degreening occurred when light was reduced to extremely low levels

Table 1. Influence of R33417 and light on preharvest degreening of regreened 'Valencia' orange fruit.

Fruit location[z]	R33417 (ppm)[y]	Fruit covered[x]	ΔA[w]
Exposed	0	No	0.02a[v]
Exposed	1000	No	0.10c
Exposed	0	Yes	0.01a
Exposed	1000	Yes	0.01a
Shade	0	No	0.01a
Shade	1000	No	0.05b
Shade	0	Yes	0.00a
Shade	1000	Yes	0.01a

[z] Fruit were either located on the outside of the tree canopy and thus directly exposed to sunlight or inside the canopy and thus received little or no direct irradiation.
[y] Fruit immersed 3 min in 95% ethanol containing 0 or 1000 ppm R33417.
[x] Individual fruit were covered with a black broadcloth bag which reduced light intensity to about 5% of that on uncovered fruit. The bags caused no detectable change in the temperature of the air surrounding the fruit.
[w] Average decrease in absorbance from the time the experiment was initiated until 6 days later. Average absorbance at day zero was 0.32.
[v] Mean separation by Duncan's multiple range test, 5%.

Table 2. Postharvest degreening of regreened 'Valencia' orange fruit. The influence of several factors on the effectiveness of R33417 as a degreening agent.

Treatment no.	Time between chemical and light exposure (hr)[z]	Length of light exposure (hr)[y]	Temp after light exposure (°C)[x]	R33417 (ppm)[w]	ΔA[v] Time after chemical was applied (hr) 24	48	72
1	4	1/4	4	0	0.01ab[u]	0.00a	0.00a
2	4	1/4	4	1000	0.02b	0.01ab	0.01a
3	4	1/4	27	0	0.00a	0.00a	0.00a
4	4	1/4	27	1000	0.02b	0.03bc	0.03ab
5	4	6	4	0	0.02b	0.01ab	0.02ab
6	4	6	4	1000	0.04c	0.03bc	0.04bc
7	4	6	27	0	0.06d	0.07d	0.07cd
8	4	6	27	1000	0.09e	0.12f	0.13e
9	4	continuous	—	0	0.09e	0.14f	0.17f
10	4	continuous	—	1000	0.13f	0.17g	0.17f
11	24	1/4	4	0	0.00a	0.00a	0.00a
12	24	1/4	4	1000	0.01ab	0.01ab	0.01a
13	24	1/4	27	0	0.00a	0.00a	0.00a
14	24	1/4	27	1000	0.01ab	0.01ab	0.02ab
15	24	6	4	0	0.00a	0.02abc	0.02ab
16	24	6	4	1000	0.01ab	0.04c	0.03ab
17	24	6	27	0	0.00a	0.04c	0.05bc
18	24	6	27	1000	0.01ab	0.06d	0.08d
19	24	continuous	—	0	0.00a	0.08de	0.14e
20	24	continuous	—	1000	0.01ab	0.09e	0.15ef

[z] Fruit in darkness at 27°C during this interval.
[y] Fruit received an irradiance of 0.43 ± 0.04 cal cm^{-2} min^{-1} at wavelengths between 400 and 700 nm. This accounted for 54% of the total irradiance. The remainder was at wave lengths greater than 700 nm. Metal halide vapor and color-improved mercury lamps were used with a ratio of input watts of 11 to 4, respectively. Air temp was 31.5 ± 0.5°C. Fruit surface temp was 42 ± 3°C. Relative humidity was 71%. All conditions refer to the top side of fruit, which was the side used for degreening studies.
[x] Fruit stored in darkness at the indicated temperatures.
[w] Fruit immersed 3 min in 95% ethanol containing 0 or 1000 ppm R33417.
[v] Average decrease in absorbance from the time R33417 was applied. Average absorbance at zero hr was 0.32.
[u] Mean separation, within columns, by Duncan's multiple range test, 5%.

Table 3. The influence of light, temperature, and R33417 on the postharvest degreening of regreened 'Valencia' orange fruit.

R33417 (ppm)[z]	Storage condition following chemical treatment	ΔA[y]					
		Time after chemical was applied (hr)					
		18	26	42	50	67	93
0	Darkness at 40°C	0.01a[x]	0.02a	0.04a	0.05a	0.06a	0.09a
1000	Darkness at 40°C	0.06b	0.06b	0.07b	0.08b	0.08a	0.10a
0	Light[w]	0.06b	0.08c	0.11c	0.12c	0.13b	0.16b
1000	Light	0.10b	0.13d	0.16d	0.16d	0.18c	0.20c
Significance of F[v]							
Storage condition (S)		***	***	***	***	***	***
Chemical (C)		***	***	***	***	***	**
S × C		NS	NS	NS	NS	NS	NS

[z] Fruit immersed 3 min in 95% ethanol containing 0 or 1000 ppm R33417.
[y] Average absorbance at zero hr was 0.33.
[x] Mean separation, within columns, by Duncan's multiple range test, 1%.
[w] Fruit received the same irradiance specified in Table 2. Air temp was 11.5 ± 0.5°C. Fruit surface temp was 29 ± 3°C. Relative humidity was 82%.
[v] NS indicates not significant at 5% level; ** significant at 1% level; *** significant at 0.1% level.

by covering fruit with black broadcloth bags (Table 1). Likewise, in the postharvest experiment, a positive correlation was obtained between length of light exposure and the extent of degreening (Table 2, compare results of treatments 4, 8, and 10). Since both of these experiments involved high light intensities, we must consider the possibility that all or part of the light effect on the activity of the chemical was due to increased rind temperatures.

Results obtained in a subsequent experiment (Table 3) show that R33417 caused some degreening in the absence of light during the first 50 hr when fruit temperature was maintained at 40°C. The effect of light on the response to the chemical was not solely due to elevated temperatures. This is shown by the fact that more chemically-induced degreening occurred in light than in the dark even when fruit temperatures were lower in the light than in the dark.

Light dependent chemical degreening was also observed with rind temperatures as low as 10°C (Table 4). In our postharvest studies, degreening occurred in the absence of R33417 when light or heat was supplied, therefore, R33417 may potentiate the destructive effects of intense light and high temperatures. This agrees with the results, but more research is needed to test this possible mode of action. The importance of light to the activity of R33417 is similar, in some respects, to the light-promoted activity of the bipyridylium herbicides (2). Whether these 2 classes of compounds share more than a superficial similarity in mode of action is unknown.

Relations between R33417 and ethylene dependent degreening were examined in a preliminary experiment. R33417 was applied as spot treatments to green-colored lemons after which the fruit were subjected to ethylene in the dark at 20°C. Surprisingly, degreening occurred in all except the chemically-treated areas; yet, R33417 will degreen detached lemons in the absence of exogenous ethylene if light is present. Thus, it appears that R33417 and ethylene have different modes of action on degreening.

Results from treatments 9 and 10 vs. 19 and 20 in Table 2 show that R33417 was less effective when the delay between treatment and exposure to light was 24 rather than 4 hr. This implies that the compound is being inactivated, but we have conducted no residue and metabolism studies and thus have no direct evidence that the compound is metabolized.

Light-induced degreening may be due to the photochemical oxidation of chlorophyll (3) since degreening took place at low temperatures (Table 4), since intense radiation was more effective than moderate or low irradiances (compare results in Tables 2 and 3 with those of Table 4, noting that differences in light quality may be responsible for differences in effectiveness), and since degreening depended on the length of exposure to light with a lag period of at least 15 min (compare treatments 3, 7 and 9 in Table 2). Dark reactions were also involved in both the chemical and light-induced

Table 4. Postharvest degreening of regreened 'Valencia' orange fruit. A study of light intensity vs. the effectiveness of R33417 as a degreening agent.

R33417 (ppm)[z]	Light intensity[y]	ΔA[x]			
		Time after chemical was applied (hr)			
		16	41	65	90
0	Low	0.01a[w]	0.01a	0.03a	0.04a
1000	Low	0.01a	0.03b	0.06b	0.08b
0	Moderate	0.01a	0.03b	0.06b	0.08b
1000	Moderate	0.01a	0.04b	0.08b	0.09b
Significance of F[v]					
Light intensity (L)		NS	*	**	*
Chemical (C)		NS	*	**	*
L × C		NS	NS	NS	NS

[z] Fruit immersed 3 min in 95% ethanol containing 0 or 1000 ppm R33417.
[y] Moderate light intensity fruit received an irradiance of 0.19 ± 0.02 and low light fruit received 0.12 ± 0.01 cal cm^{-2} min^{-1} at wavelengths between 400 and 700 nm. This accounted for 60% of the total irradiance. Color-improved mercury lamps were used. Air temp was 5.8 ± 0.2°C and relative humidity was 88% in all treatments. Fruit surface temps were 12 ± 2°C and 10 ± 1°C for moderate and low light fruit, respectively.
[x] Average absorbance at zero hr was 0.34.
[w] Mean separation, within columns, by Duncan's multiple range test, 5%.
[v] NS indicates not significant; * significant at 5% level; ** significant at 1% level.

reductions in chlorophyll content. Fruit treated with R33417 and/or light were stored in the dark at 4 and 27°C; more degreening was observed with fruit stored at the higher temperature (Table 2, compare treatment 6 with 8, and treatment 5 with 7).

Significant direct effects of light on degreening were not observed preharvest (Table 1) indicating that attached fruit may respond differently than excised fruit, or that the lamps used in our postharvest studies were more effective in degreening than solar radiation. Degreening of citrus leaves has not been observed with seedlings that have been subjected to the light and temperature treatments used in the postharvest experiments, indicating that citrus fruit are much more susceptible to loss of chlorophyll than are attached leaves.

The horticultural implications of R33417 and light-induced degreening are not clear. Rapid and uniform degreening of regreened 'Valencia' oranges from preharvest applications would be of potential value, especially if storage and shelf-life problems are not aggravated. When 1000 ppm R33417 was sprayed on leaves and fruit in a preliminary look at this possibility, leaf abscission occurred. Thus, experiments designed to study the practical value of R33417 as a

preharvest degreening agent should include evaluations of leaf abscission. Commercial use of light as a degreening agent is limited by the relatively high irradiances required for the effect and by the cooling system needed to prevent rind scald at these irradiances.

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Effect of Magnesium Sources and Rates on Correction of Acute Mg Deficiency of Pecan[1]

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Abstract. Magnesium sulfate applied as a soil amendment (34 kg Mg/ha annually for 3 years or a single application of 224 kg Mg/ha) increased leaf Mg 5 years after initial application. Dolomite increased soil pH and soil test Mg but not leaf Mg. Sulfate of potash magnesia and MgO increased soil test Mg and slightly, though insignificantly, increased leaf Mg. Single foliar sprays of $MgSO_4$ and $Mg(NO_3)_2$ did not affect leaf Mg.

Magnesium deficiency is a serious problem in pecan (*Carya illinoensis*, Koch.) orchards of the southeastern U. S. (2, 16). In 1967, almost half of the leaf analysis samples in Georgia contained less than 0.3% Mg (16). Many of these exhibited the characteristic Mg deficiency symptoms described by Sharpe et al. (12).

Efforts to correct Mg deficiency have given erratic results. Sharpe et al. (11) applied sea water magnesia (80–90% MgO derived from sea water) to soil under pecan trees at rates of 0–5.4 kg/tree and obtained no yield increase, but it increased foliage Mg 7 years after treatments began. They also (12) reported that $MgSO_4$ improved foliage color of 'Moore' in one year, but 'Moneymaker' responded less rapidly. Others have reported that either leaf Mg content, yield, or foliage appearance was improved with $MgSO_4$ for citrus (4, 5, 10) and apple trees (3). Other materials sometimes effective as Mg sources when used as soil applications were MgO (3, 10), sulfate of potash magnesia (10), kieserite, $MgCO_3$, and dolomite (3); however, Ford (6) obtained no increase in growth or leaf Mg content and no reduction of Mg deficiency symptoms with soil applications of $MgSO_4$ to apple rootstock beds. Similar results were obtained by Woodbridge (15) for apple.

Foliar sprays are sometimes used to prevent or correct Mg deficiency symptoms. Epsom salt sprays were effective on apple (3, 6, 15), but Calvert (personal communication) reported poor results on citrus.

Since Mg deficiency is a serious problem in many pecan production areas, a test was initiated in 1968 to determine best methods for correcting low tissue levels of Mg.

Materials and Methods

An orchard of over 100 mature pecan trees spaced 18 × 18 m apart located in Pierce County, GA was selected for the test. The owner reported that the orchard produced high yields of high quality pecans prior to developing a condition later identified as severe Mg deficiency. Trees had been in a state of decline for several years prior to the initiation of the test. Dieback in the tops resembled Zn deficiency; however, leaf analyses revealed extremely low Mg and high Zn. Leaf samples in 1965 revealed that the Mg concn was .05%, which is lower than the .08% level obtained by Alben (1) when he grew pecans in sand culture without Mg. Leaves showed characteristic Mg deficiency symptoms described by Sharpe et al. (12). Treatments (Table 1) were replicated 5 times on 'Moneymaker' at one end of the grove, and 5 times on 'Stuart' at the other end in a randomized complete block design with single tree plots. All treatments, except the spray treatments and the check, were broadcast evenly underneath the trees in early spring. Dolomite (10% Mg) was applied at 224 kg Mg/ha (1 ton/acre) in winter of 1968 and again in 1969. Magnesium sulfate was applied at the rate of 224 kg Mg/ha as a single application in 1968 only and at annual rates of 34 kg Mg/ha for 3 years. Magnesium from K_2SO_4-$2MgSO_4$ (sulfate of potash magnesia) and MgO was also applied annually at 34 kg/ha. This rate is often used for agronomic crops. Since K has been shown to reduce Mg uptake (13), an additional treatment of K_2SO_4 was included, which supplied the same rate of K as K_2SO_4-$2MgSO_4$, to determine detrimental effects of K on Mg uptake. Raplex Mg, a by-product of the paper industry containing 4.1% Mg. was used at the rate of 34 kg of product/ha. Magnesium sulfate and $Mg(NO_3)_2$ sprays at equivalent Mg levels were applied to the point of run-off when leaves were approx ½ matured each spring. An additional treatment of Claw-El Mg (Brandt Chemical Co., 4% Mg) foliar spray was applied to 15 trees at 30 g product/l in 1969 and to 7 trees at 6 g product/l in 1970 as described for the other sprays but had no effect on the parameters studied and will not be discussed here. All treatments were discontinued after 1970. The grove received a uniform application of N (112 kg/ha) each winter. A subsoil furrow was cut approx 46 cm deep 9 m from each side of each tree to reduce root crossfeeding in 1968 and 1969. In 1970 a trench was cut 127 cm deep, which extended well through the root zone. Vigor ratings (0 = poor; 9 = excellent) were made in August 1968 and 1969, and terminal shoot growth ratings were made at a height of approx 7 m in 1968, 1969, and 1970. Magnesium deficiency ratings (0 = no symptoms; 5 = severe leaf symptoms) were made at the same time.

Soil samples from the top 15 cm were taken under each tree during the winter each year to determine residual effects of treatments. The pH was determined from a 1:1 (soil:water) solution. Soil P, K, and Mg were determined from a 0.05 N HCl-0.025 N H_2SO_4 extract of the soil. Phosphorus was determined colorimetrically by developing the blue molybdophosphoric acid color. Potassium was determined from the extract by flame photometry and Mg was determined in 1970 only by atomic absorption spectrophotometry. Nematode assays were made from the soil samples collected February 19, 1970 by the sugar flotation method described by Jenkins (8) to determine whether these parasites affected results.

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