The Possible Cause of Red Leaf Disease and its Effect on Barlinka Table Grapes*

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The so-called red leaf (RL) phenomenon of Barlinka table grapes was investigated in the Hex River Valley, De Doorns. The symptoms of RL were found to resemble those of grapevine leafroll (GLR) disease to a certain extent, but also differed markedly from GLR in that initial reddening occurs in veins of apical leaves, with the typical downward curling of affected leaves not a distinctive feature. Like GLR, RL induces uneven and retarded ripening, small and shot berries, without colour in extreme cases, making the grapes worthless for export. Vine vigour is also seriously reduced. The cause of RL was found not to be a Ca deficiency as previously postulated and generally accepted. Foliar and soil applied Ca proved ineffective. Reciprocal grafting of red leaf-affected and heat-treated plant material showed that RL is associated with plant-transmissible pathogens, probably similar or belonging to viruses associated with GLR.

The late maturing, black, table grape variety, Barlinka, was brought from northern Africa to South Africa during 1910 (Perold, 1926). According to unpublished KWV (Ko-operatieve Wijnbouwers Vereniging) statistics, it occupied 31% of the total area of about 7 000 ha under table grapes in 1987, the bulk of this (83%) in the semi-arid Hex River Valley, about 120 km north-east of Cape Town.

The first appearance of the so-called red leaf (RL) symptom on black table grape vines is not recorded, but it was apparently already known during the sixties to affect individual vines or patches in especially Barlinka vineyards in the Hex River Valley. This phenomenon has received concerted attention from researchers only since 1971, after urgent requests from producers in whose vineyards the occurrence of RL was extensive. That same year Blommaert (1971) reported results of a preliminary survey, the main conclusion being that affected vines had sub-normal concentrations of calcium and magnesium in leaf blades sampled at véraison (colour break). It was postulated that the previous abnormally dry seasons may have caused low nutrient reserves in vines, resulting in inadequate root activity and consequently low Ca uptake. The possibility that one or more viruses may have contributed to RL was also mentioned.

Blommaert *et al.* (1973) described RL as a red to purple discolouration of leaves of red varieties, especially Barlinka and Alphonse Lavallée. It is similar in appearance to natural autumn colours but develops abnormally early in the season (beginning to mid-January). Affected vines have low vigour and produce a poor crop, often not suitable for export. A follow-up survey revealed that RL vines have poorly developed root systems and low Ca and Mg, but also low N concentrations in leaf blades (Blommaert *et al.*, 1973). It was concluded that the increasing use of high analyses NPK fertilisers, i.e. low in Ca, led to poor Ca nutrition and consequently poor root development, which induced the low Mg and N uptake. This result was accepted by the industry and calcium nitrate foliar sprays were recommended as a remedy against RL.

In a review of the physiological effects of Ca on plants, Bangerth (1979) listed four biological functions of Ca, *viz.* its effect on membranes, enzymes, cell walls and phytohormone interactions. Active root growth is necessary for sufficient Ca uptake, but to ensure good root growth a high Ca concentration in the soil solution is required.

Red colouration of basal leaves is also associated with extremely acid soils. However, this symptom is probably a complex of Ca Mg deficiencies as well as aluminium and manganese toxicities, caused by low soil pH, and is not a specific Ca deficiency symptom. Locally the white wine variety Hárslevelü was also observed to be very sensitive to high soil acidity, expressing similar 'acidity complex' symptoms, in this case yellow colouration, and an extremely low Ca concentration in leaf blades: 0,64% Ca compared to 1,52% in leaf blades from adjacent, symptomless vines. According to Delmas (1971) and Winkler *et al.* (1974), Ca deficiency symptoms have not yet been found under field conditions in vines. Symptoms induced in vines grown in Ca-deficient growth media showed marginal and interveinal chlorosis, followed by marginal necrotic spots that expand interveinally (Delmas, 1971). Yet no obvious Ca-deficiency symptoms could be induced in Chenin blanc vines grown in sand culture at Nietvoorbij, but Ca-deficient vines tended to die off during dormancy (W.J. Conradie, 1981 – personal communication).

Goheen & Cook (1959) were the first to relate the leaf discolouration phenomenon called 'red leaf' in California to *rougeau, flavescence* or *brunissure* of vines in European countries and identified it as caused by grapevine leafroll (GLR) viruses. According to Goheen & Cook (1959),

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Ravaz & Roos speculated in 1905 the *rougeau* in France was the result of nutrient deficiencies, especially Ca, but that later foliar analyses by Ravaz and colleagues in 1933 showed that this phenomenon was associated with K deficiencies. Low K concentrations in leaves were subsequently confirmed by Goheen & Cook (1960) and Millikan, Pickett & Hemphill (1963) as a characteristic of GLR. Although GLR leaf symptoms resemble those of Mg deficiencies, the former can be distinguished from the latter by their normal Mg content (Goheen & Cook, 1960). GLR symptoms for red varieties were described by Bovey *et al.* (1980) as reddish spots, appearing on basal leaves from mid-summer, which coalesce until the whole surface becomes red, while the primary and secondary veins and immediately adjacent tissue remain green. The blades thicken and roll downwards. In advanced stages necrotic areas may develop. Berries mature unevenly and later, usually with a lower sugar concentration compared to that of healthy vines.

In an irrigation and fertilisation trial in a Barlinka vineyard in the Hex River Valley, the presence of RL was noted soon after the vineyard was established in 1979. Eventually it was determined that about 28% of the data vines were affected. The obvious negative effects of RL on bunch quality and vine vigour as well as on the nutient concentrations in leaves necessitated the elimination of data gathered from all vines with a history of RL. This paper reports on further investigations, aimed at establishing the nature and cause of RL and at finding solutions to the problem.

MATERIALS AND METHODS

The occurrence and severity of RL were evaluated at harvest for five seasons from 1983/84 for all 1 440 data vines of an irrigation/fertilisation trial with Barlinka on the Nietvoorbij Institute for Viticulture and Oenology (Nietvoorbij) experimental farm at De Doorns in the Hex River Valley. A scale of 1 (no visible symptoms) to 4 (severely affected) was used. These data were processed, using Freedman's analyses of variance by ranks (Siegel, 1956).

To verify the results of Blommaert *et al.* (1973) 20 leaf laminae and petioles opposite bunches were sampled at pea size and véraison during the 1985/86 season from each of 15 randomly selected symptomless vines as well as from 15 vines with definite red leaf symptoms, and chemically analysed. The effect of RL on bunch and berry quality was evaluated at harvest during 1988 by sampling three representative bunches from each of two randomly chosen RL classified vines, from two normal vines with badly coloured grapes and from two normal vines with well coloured grapes. These bunches were analysed for berry mass, bunch density, and sugar and acid concentration.

To verify the hypotheses that RL is caused by a Ca deficiency, the Ca content of soil sampled in 1990 from the 24 treatment combination plots (samples were pooled over crop load and replicates), which included a 2 t ha^{-1} dolomitic lime treatment, was correlated with the mean RL index of corresponding vines. On a smililar basis, correlations were also calculated for soil Ca content and that of leaf blades and petioles.

To verify the effectiveness of Ca foliar sprays in alleviating RL, vines with a history of red leaf symptoms were randomly selected: 15 vines on plots that received 2 t ha^{-1} dolomitic lime during 1987 and 15 vines on unlimed plots. These vines were grouped into 5 blocks and the following treatments applied during the 1990/91 and 1991/92 seasons: 1 = No foliar spray (control), 2 = Three Ca-nitrate foliar sprays at a concentration of 65 g dm^{-3} and 3 = Three urea foliar sprays at a concentration of 17 mg dm^{-3}.

The first sprays were applied at 40-50 cm shoot length and repeated at 3-weekly intervals. The urea sprays were included to evaluate possible response to the N present in the calcium nitrate sprays. Bunch and shoot mass were recorded for each vine and the incidence of RL (1 to 4 scale as described) and bunch colour (scale of 1 (black) to 6 (green)) scored. Leaf blade and petiole samples were collected from each vine at pea-size stage.

All leaf samples were washed, dried in a forced draft oven at 70 °C and analysed by Elsenburg Agricultural Development Institute (EADI). After dry ashing, P, B and cations were determined in a hydrochloric acid background solution with direct current plasma emission (DCP) spectrography and N by the macro Kjeldahl process. Soil samples were air dried, passed through a 2 mm diameter sieve and analysed at EADI, using atomic adsorpsion spectrometry (AA) for the determination of 0,05 M $SrCl_2$ extracted Ca.

To investigate the possibility of a pathogen being responsible for RL, a factorial, randomised, block-design, pot experiment, with two growth media and four reciprocal graft combination treatments and five replicates, was started in 1985. The grafted vines were grown for two seasons in glazed, earthenware, 38 cm inner diameter (48 dm^{-3}) pots. Treatments were as follow: (A) Growth medium: 1= Soil from the Hex River Valley irrigation/fertilisation experiment vineyard, 2= Acid washed, coarse sand and/perlite mix (50:50) and (B) Graft combination: 1= Heat-treated, 'virus free' Barlinka clone 47, grafted on the same heat-treated material as 'rootstock' (H/H), 2= Heat-treated, 'virus free' Barlinka clone 47, grafted on RL classified Barlinka clone 47 material from the fertilisation trial as 'rootstock' (H/R), 3= RL classified Barlinka clone 47 material from the fertilisation trial, grafted on heat-treated Barlinka clone 47 material as 'rootstock' (R/H), 4= RL classified Barlinka clone 47 material from the fertilisation trial, grafted on the same RL material as 'rootstock' (R/R).

The sand/perlite pots received 2 dm^{-3} Hoagland nutrient solution (Hoagland & Arnon, 1950) on a weekly basis, whereas the pots containing Hex River Valley soil received the equivalent of 120 kg N ha^{-1}, split into three equal increments at bud burst, flowering and véraison. During April 1986 ten leaves were sampled from the top third of shoots of each vine and evaluated for red leaf symptoms, using a 1 (no symptoms) to 5 (severe symptoms) intensity scale. During autumn 1987 RL severity was photographically recorded. Shoot mass was measured at pruning in August 1987 and 1988.

RESULTS AND DISCUSSION

Visual symptoms of RL differ markedly in certain aspects from those of GLR-affected vines. The first appearance of GLR discolouration is on basal leaves, while apical leaves of RL vines redden first. Whereas leaf veins and immediately adjacent areas that stay green is a typical symptom of GLR, the veins of RL leaf blades redden first, without the typical downward curling of GLR. This reddening developed over the whole leaf surface and spread towards older leaves, finally giving the whole vine a distinctive yellowish-red appearance. Leaves with RL symptoms also have a smaller angle between the abaxial plane of the blade and the petiole than non-affected leaves (Fig. 1). Similar symptoms have been reported for water stress (Smart, 1974) and P deficiencies (Bouard & Pouget, 1971). Apparently this symptom has not been reported by GLR.

Analyses of variance of ranked RL indices for five seasons showed no significant effects of two levels of P+K fertilisation, two different seasonal patterns of N application and three levels of N application on the severity of RL (data not shown). Although crop load significantly affected RL severity during two seasons (Fig. 2), this appears to be incidental because of the inconsistent and illogical pat-

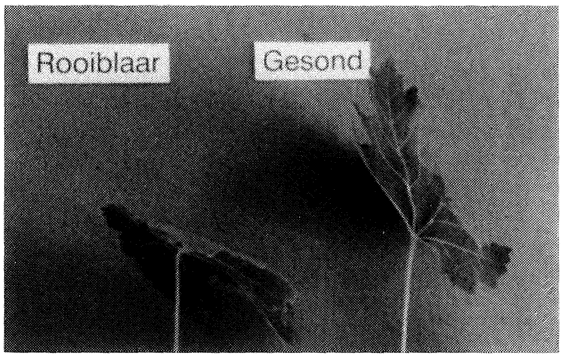

FIGURE 1
Reduced angle between petiole and abaxial plane of Barlinka leaf with Red Leaf symptoms (left), compared with that of normal leaf (right).

TABLE 1
The effects of Red Leaf on bunch quality of Barlinka table grapes: Hex River Valley, 1988.

Bunch category	Bunch mass (g)	Berries /bunch	Berry mass (g)	Grape stem length (cm)	Bunch density *	Sugar (^0B)	Acid $(g\ dm^{-3})$	pH
Red Leaf	317a	89a	3,8a	44,5a	7,2a	10,5a	5,5a	3,37a
Green bunches	576b	105a	5,6a	50,4a	11,3a	15,1a	4,5a	3,48a
Black bunches	669b	100a	6,7b	47,7a	14,0b	18,5b	4,2a	3,60b

* Estimated as (berry mass per bunch/total bunch stem length).
a,b: Means in columns followed by the same symbol do not differ significantly at 95 % confidence level.

TABLE 2
Effect of Red Leaf on the nutrient content of Barlinka leaf blades: Hex River Valley, 1985/86.

Stage	Category	Element											
		N	P	K (%)	Ca	Mg	Na	Cl	Fe	Mn (mg kg^{-1})	Zn	Cu	B
Pea size	Red Leaf	2,67	0,40	1,08	1,29	0,28	0,03	338	61	177	23	5,0	36
	Normal	2,75	0,38	1,28	1,60	0,34	0,04	374	82	261	32	5,2	45
Significance		ns	ns	***	***	***	***	**	***	***	***	ns	***
Colour break	Red Leaf	2,19	0,30	0,92	1,48	0,29	0,042	531	77	220	36	8,5	31
	Normal	2,36	0,26	1,14	1,89	0,34	0,043	614	97	328	41	8,7	35
Significance		ns	ns	***	***	***	*	**	***	***	**	ns	ns

ns: non-significant; *: $p \leq 0,05$; **: $p \leq 0,01$; ***: $p \leq 0,001$.

TABLE 3
Effect of Red Leaf on the nutrient content of Barlinka petioles: Hex River Valley, 1985/86.

Stage	Category	Element											
		N	P	K (%)	Ca	Mg	Na	Cl	Fe	Mn (mg kg^{-1})	Zn	Cu	B
Pea size	Red Leaf	1,01	0,54	2,05	1,06	0,34	0,04	1316	26	140	38	8,0	29
	Normal	0,86	0,53	2,78	1,17	0,40	0,05	1477	30	199	41	5,3	32
Significance		*	ns	***	**	***	*	*	ns	***	ns	**	***
Colour break	Red Leaf	0,63	0,48	1,92	1,18	0,46	0,05	2208	34	268	58	5,0	24
	Normal	0,64	0,60	3,52	1,59	0,57	0,06	2856	41	436	73	6,0	29
Significance		ns	**	***	***	***	***	***	***	***	***	**	***

ns: non-significant; *: $p \leq 0,05$; **: $p \leq 0,01$; ***: $p \leq 0,001$.

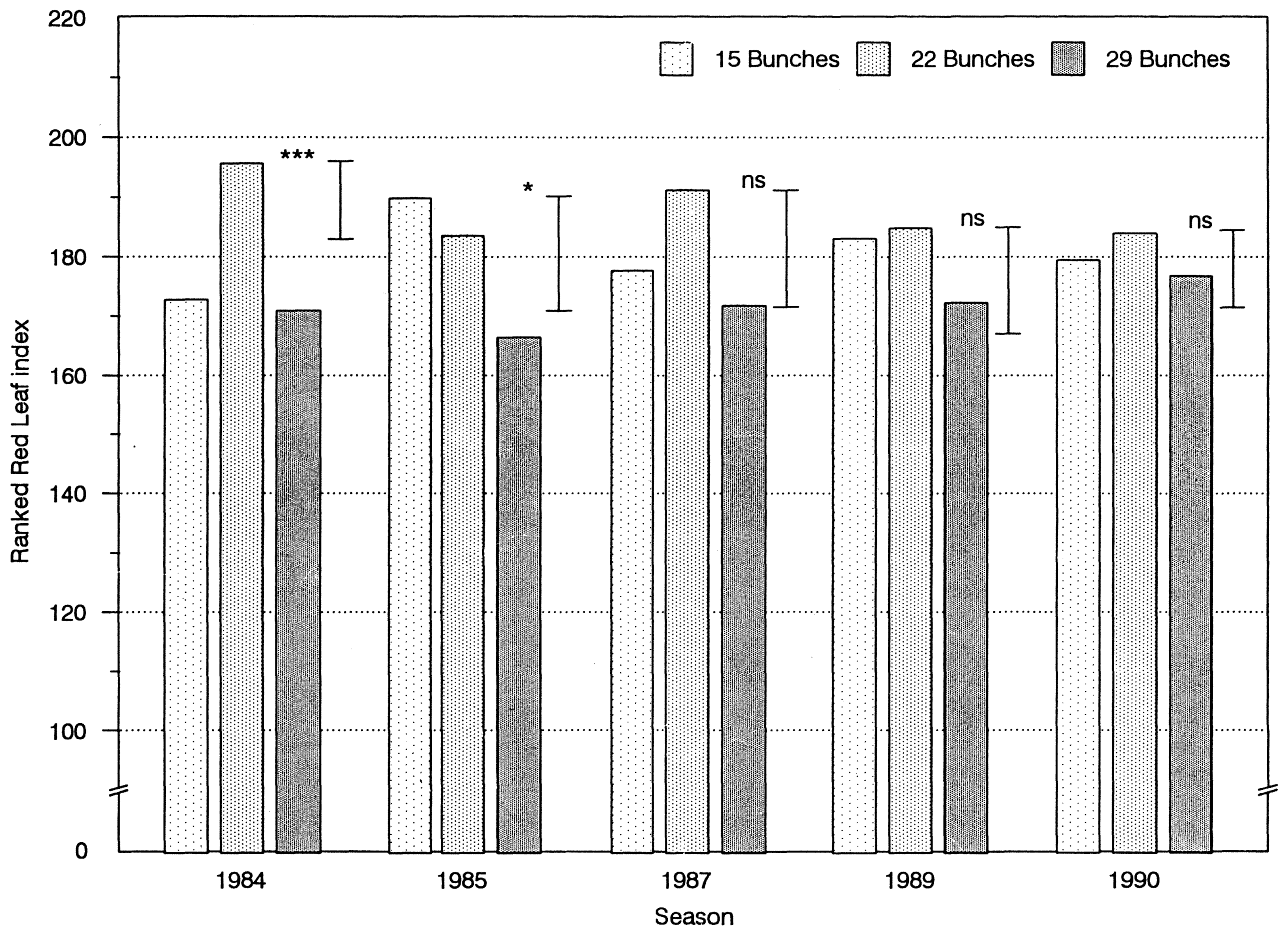

FIGURE 2

Effect of crop load (bunches vine^{-1}) on the severity of Red Leaf symptoms of Barlinka vines in an irrigation/fertilisation trial, Nietvoorbij Experimental farm, Hex River Valley.

tern of this variable. There were no differences between ranked seasonal means of RL, implying that RL did not spread or increase in severity over years.

Similar to GLR, RL has a pronounced effect on grape quality, as illustrated in Table 1. Red Leaf reduces the size of bunches and berries, resulting in loose, shot-berried bunches with virtually no colour in extreme cases and totally worthless for marketing. Like GLR, RL not only retards but even prevents maturation, resulting in low must sugar concentrations and pH.

Leaf blades of RL vines at pea size and at véraison had, except for N, P and Cu, significantly lower concentrations of all the other nutrients analysed, the Ca concentrations at véraison being below the minimum norm of 1,6% (Beyers, 1962), compared to those of healthy vines (Table 2). Petiole analyses largely confirmed these results, RL petioles have extremely low K concentrations at véraison (Table 3). Low K concentrations in RL blades, which conforms to those of GLR (Millikan *et al.*, 1963), were not found by Blommaert *et al.* (1973) and the negative correlation of N with degree of RL found by them was not confirmed by this study. The significance of the generally low element concentrations in RL material is not clear, but may point to some form of blockage in the uptake or transport systems.

No significant relationships between the Ca content of soil samples (50% of the plots sampled received dolomitic lime) and RL severity, nor between the Ca content of soil and that of leaf blades or petioles, could be determined (data not shown). Soil samples taken at the end of the 1989/90 season from the 0-300 mm topsoil layer of limed plots had almost double the Ca content compared to similar samples from unlimed plots (1,12 cmol (+) kg^{-1} compared to 0,65 cmol (+) kg^{-1}). This indicated that RL is not caused by the Ca concentration in soil. The vine performance data for limed and unlimed plots (Table 4) support this finding.

Calcium foliar feeding also had no effect on the crop, shoot growth or the incidence of RL (Table 4). Although Ca or urea sprays slightly improved the colour of bunches during the first season of application, this was not confirmed during the second season.

The Ca concentration in leaf blades was increased by Ca foliar sprays during the first season of application, but not during the following season (Table 5) and remained below the minimum norms of 1,2% and 1,5% at fruit set and véraison respectively, as proposed by Conradie (1986). The concentration of K, Fe, Cu and B in leaf blades was also significantly enhanced by Ca foliar sprays during the first season, while the Mg concentration in leaf blades was similarly increased by urea sprays. However, the magnitude of all these increases was small and probably has few practical implications. Even on plots that received 2 t ha^{-1} dolomite lime during April 1987, no improvement in Ca or Mg contents of leaf blades was found. Petiole analyses appeared to be even more insensitive to the Ca treatments and only at vérasion showed Ca concentrations lower than the minimum norm proposed by Conradie (1986) (data not shown).

These findings showed that, although the soil Ca content could be improved by liming, this apparently did not improve Ca uptake by RL vines, neither could this be successfully rectified by foliar sprays. It is therefore doubtful that Ca is the causative agent of RL.

The reciprocal grafting pot experiment indicated that RL was caused by a plant material transmissible pathogen. During the first season all grafts that had a component classified as RL expressed red leaf symptoms, which were aggravated when vines were planted in a sand/perlite medium (Table 6). Shoot growth was drastically inhibited during the first season by the Hex River Valley soil potting medium. The sand/perlite medium induced similarly poor growth the following season. The mean shoot growth was significantly better during the first season when RL material was combined with heat-treated material in the graft combination, but this was not evident the next season when limited shoot growth was experienced for both media.

The retarded growth in the Hex River Valley soil medium, which was not sterilised, may be attributed to the

TABLE 4
The effect of Ca foliar sprays on the performance of and severity of Red Leaf in Barlinka, Hex River Valley.

Season	Treatment	Property measured					
		Bunches/ vine	Crop mass/ vine (kg)	Bunch mass/ (kg)	Shoot mass/ vine (kg)	Bunch colour[1]	Red Leaf index[2]
1990/91	Unlimed soil	17,3a	5,62a	0,30a	1,29a	5,1a	3,4a
	Limed soil	15,1a	4,29a	0,26a	1,08a	5,3b	3,7a
	Control	15,9a	4,81a	0,27a	1,08a	5,5b	3,6a
	Ca sprays	13,6a	3,84a	0,26a	1,12a	5,0a	3,6a
	Urea sprays	19,1a	6,22a	0,31a	1,35a	5,2a	3,5a
Seasonal mean		16,2	4,96	0,28	1,18	5,1	3,6
1991/92	Unlimed soil	12,5a	4,39a	0,34a	1,84a	4,30a	
	Limed soil	14,5a	4,63a	0,27b	1,22b	4,60a	
	Control	12,3a	4,63a	0,32a	1,34a	4,57a	
	Ca sprays	11,7a	3,83a	0,29a	1,35a	4,21a	
	Urea sprays	16,4a	5,33a	0,31a	1,91a	4,57a	
Seasonal mean		13,5	4,51	0,31	1,53	4,45	

[1] Colour scale from 1 (black) to 6 (almost totally green).
[2] Scale from 1 (normal) to 4 (severely affected).
a,b: Groups of means in columns followed by the same symbol do not differ significantly at 95% confidence level.

TABLE 5
Effect of soil and foliar-applied Ca on element concentration at pea-size stage in blades of Red Leaf-affected Barlinka vines; Hex River Valley.

Sampling date	Treatment	Element									
		N	P	K (%)	Ca	Mg	Fe	Mn	Zn ($mg\ kg^{-1}$)	Cu	B
05/12/1990	Unlimed soil	2,19a	0,24a	1,06a	1,02a	0,24a	66a	190a	29a	8,5a	23a
	Limed soil	2,14a	0,23a	1,01b	1,00a	0,24a	66a	166a	30a	8,2a	23a
	Control	2,22a	0,22a	1,03a	0,95a	0,23a	61a	173a	28a	7,5a	20a
	Ca sprays	2,17a	0,26a	1,09b	1,10b	0,23a	72b	180a	31a	9,7b	27b
	Urea sprays	2,11a	0,22a	0,99a	0,98a	0,25b	65a	181a	30a	7,8a	22a
Period mean		2,17	0,23	1,04	1,01	0,24	66	178	30	8,3	23
18/12/91	Unlimed soil	2,22a	0,18a	1,00a	0,81a	0,21a	79a	182a	26a	16,7a	23a
	Limed soil	2,05a	0,18a	1,00a	0,79a	0,21a	81a	154a	26a	17,2a	24a
	Control	2,03a	0,17a	0,97a	0,80a	0,21a	72a	179a	25a	17,6a	23a
	Ca sprays	2,23a	0,19a	1,00a	0,78a	0,20a	89a	146a	27a	16,0a	27a
	Urea sprays	2,15a	0,17a	0,95a	0,82a	0,21a	79a	178a	27a	17,3a	21a
Period mean		2,13	0,18	0,98	0,80	0,21	80	167	26	17,0	24

a,b: Groups of means in columns followed by the same symbol do not differ significantly at 95% confidence level.

presence of pathogens in the soil and poor resistance of own rooted Barlinka to these pathogens. In practice it is known that the gray, sandy soil of the Hex River Valley, that had been planted to Jacques rootstock (*Vitis aestivales x V. cinerea x V. vinifera*), known to have poor resistance to phylloxera and root knot nematodes (Anon, 1984), cannot be replanted with Jacquez without specific soil treatment. The soil used in the pot experiment was previously planted to Jacquez, therefore an even worse result can be expected if replanting is done with still less resistant ungrafted Barlinka material, as was apparently the case in the pot experiment. The almost equally weakened growth of vines in the sand/perlite mix the second season may be attributed to nematode infestation of this medium and/or a pot-bound situation of the two-year-old vines.

Red leaf was not scored the second season but photographs taken clearly illustrate the marked reddening caused by RL graft combinations (Fig. 3). The absence of reddening of the healthy graft combination in the Hex River Valley soil-growth medium again indicated that RL is not caused by Ca-deficient soil.

CONCLUSIONS

The extremely deleterious effect of RL on the performance of Barlinka, which still comprises more than 25% of the South African table grape export crop, is of grave concern. Based on the evidence gathered in this study, the generally accepted postulate that RL of Barlinka is caused by a Ca deficiency has to be rejected. Abnormalities in leaf content of Ca or other elements, especially low K concentration which resembles that of GLR leaves, must be ascribed to a disrupted absorption and/or transportation system caused by RL, *i.e.* the result and not the cause of RL. It also appears that the use of soil-applied dolomite and/or Ca foliar sprays to alleviate the symptoms is ineffective. This further supports the contention that Ca deficiencies are not the cause of RL.

Red Leaf is quite distinguishable from GLR. Symptoms of the former start appearing on apical leaves, first showing reddening of veins, with a reduced angle between the petiole and abaxial plane of leaves and without the characteristic GLR down and inward curling of leaf blades. In

FIGURE 3

Reddening (dark foliage) caused by the presence of Red Leaf (RL)-affected Barlinka material in reciprocal graft combinations. Graft combinations from left to right: Healthy/Healthy, RL/Healthy, Healthy/RL and RL/RL. (a) Hex River Valley soil, (b) Sand/Perlite mix.

TABLE 6
The effect of phytosanitary status of plant material on the occurrence of Red Leaf and performance of Barlinka grown in pots: Nietvoorbij.

		Treatment							
Property measured	Season	Growth medium			Graft combination				
		Sand/ perlite	Hex River soil	Significance	H/H	H/R	R/H	R/R	Seasonal mean
Red leaf severity[1]	1986	3,9	3,2	*	2,2a	3,7b	4,2b	3,9b	3,5
Total shoot mass/vine (g)	1986	264	31	***	107a	158bc	188c	138ab	148
Total shoot mass/vine (g)	1987	40,8	32,6	*	38,7b	26,6a	44,3b	37,2b	36,7

H = healthy, i.e. heat-treated material; R = red leaf classified material.
[1]Scale of 1 (no symptoms) to 5 (very severe).
a,b,c: Groups of means in rows followed by the same symbol do not differ significantly at 95% confidence level.
ns: non-significant; *: $p \leq 0,05$; **: $p \leq 0,01$; ***: $p \leq 0,001$.

view of the lack of relationships found between Ca supply and the occurrence of RL, as well as of evidence gained by reciprocal grafting of RL-affected and heat-treated plant material, it is postulated that RL is caused by one or more plant transmissible pathogen(s), possibly similar or belonging to the group of GLR-associated viruses. This aspect should be further investigated by virologists.

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