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Field experiments on the chemical control of blackcurrant reversion virus and its gall-mite vector (*Phytoptus ribis* Nal.)

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SUMMARY

Healthy blackcurrant bushes and others infected with reversion virus were exposed equally to infestation by the gall-mite vector (*Phytoptus ribis* Nal.) spreading from unsprayed sources planted nearby. Four applications of 1·0% lime sulphur at fortnightly intervals during the dispersal period were less effective than 0·05% endosulfan in preventing the infestation of healthy bushes, whereas endrin at 0·04% gave almost complete control. All spray treatments were much less effective when applied to the virus-infected bushes, although endosulfan remained superior to lime sulphur and inferior to endrin. Infected bushes sprayed with lime sulphur developed more galls than unsprayed healthy bushes.

In another experiment, four applications of endrin or lime sulphur decreased the spread of mites and reversion virus from infested bushes to adjacent sprayed and unsprayed bushes. Lime sulphur was much less effective than endrin in preventing infestation with mites, but more effective in decreasing the incidence of virus.

These results are discussed in relation to commercial spray programmes and to the design of experiments on the effectiveness and mode of action of spray materials against mites and virus.

INTRODUCTION

The gall mite *Phytoptus ribis** Nal. is a widespread pest of the blackcurrant (*Ribes nigrum* L.) and infestations have been reported in many countries. Apical and axillary buds may be infested early in the growing season and eventually become rounded galls that fail to initiate flowers or leaves. Mites also lead to indirect damage by transmitting reversion virus, which causes the most important disease affecting the crop in Britain and elsewhere.

The extensive literature on the chemical control of blackcurrant gall mite has been reviewed recently (Thresh, 1966*a*). Relatively little attention has been given to the effects of acaricides on the spread of reversion virus. Indeed, the incidence of reversion has usually been ignored in spraying experiments and the health of experimental bushes has seldom been standardized or recorded. This imposes serious limitations on the interpretation and practical relevance of the results obtained, because virus spread in other crops is not always checked by controlling the vector (Broadbent, 1957).

* Referred to as *Eriophyes ribis* (West.) Nal. in early publications and now sometimes considered to be *Cecidophyopsis ribis* (West.).

Moreover, infection with reversion virus greatly increases the susceptibility of bushes to dispersing mites and the direct damage they cause is less important than their role as vectors of the virus (Thresh, 1967). For these reasons both healthy and virus-infected bushes should be used for a full evaluation of spray measures, as in the first of the experiments now reported.

The second experiment was designed to estimate the relative effectiveness of different spray materials in destroying mites at source or after they had dispersed to sprayed and unsprayed bushes nearby.

MATERIALS AND METHODS

The acaricide experiment with healthy and virus-infected bushes, in 1966

Sprayed and unsprayed plots were replicated five times in randomized blocks. Each plot consisted of two subplots comprising four healthy bushes (var. Seabrook's Black) and four infected with reversion virus (subsequently referred to as reverted bushes). These bushes were 2 years old and free of mites when planted 2 ft apart in a single row, with older unsprayed sources of mites at the ends of each plot. The infested bushes were similar to those used previously (Thresh, 1966*b*, 1967). They were left unpruned, whereas the bushes exposed alongside were cut to ground level immediately after planting in February 1966.

The four treatments were: (1) unsprayed controls; (2) lime sulphur at 1% with 0·01% dinonyl sodium sulphosuccinate as wetter; (3) endrin at 0·04% with wetter in the formulation; (4) endosulfan at 0·05% with wetter in the formulation.

Each material was applied four times to run-off with a hand-operated 'Solo' sprayer starting on 5 April, a week after the unpruned bushes began to flower. It was intended to apply subsequent sprays at fornightly intervals as recommended for nursery bushes. Because of rain the applications were made on 21 April, 9 May and 23 May.

The experiment on spread to and from sprayed bushes, in 1965

There were five randomized blocks with four main and three subplot treatments. Each plot consisted of thirty-six healthy 'trap' bushes var. Cotswold Cross that were not infested at the outset. They were arranged on each side of six reverted bushes that provided the immediate source of virus (Fig. 1). The sources were: A, not infested with mites at planting and not sprayed subsequently; B, heavily infested at planting and not sprayed; C, heavily infested and sprayed with lime sulphur; D, heavily infested and sprayed with endrin.

The healthy bushes exposed in each plot formed an array of four 3×3 Latin squares, with twelve single bush replicates of the three subplot treatments: O, unsprayed; L, sprayed with lime sulphur; E, sprayed with endrin.

The chemicals were applied on 22 April, 30 April, 10 May and 20 May, using the same method and rates as in the previously described experiment.

The trap bushes were 2 years old when planted in February 1965 and they were pruned immediately almost to ground level. The rows ran north to south and they were 6 ft apart, with 2 ft between adjacent bushes within rows. A guard row of unpruned, uninfested blackcurrant bushes divided the experiment into two halves and pairs of gooseberry bushes separated each plot.

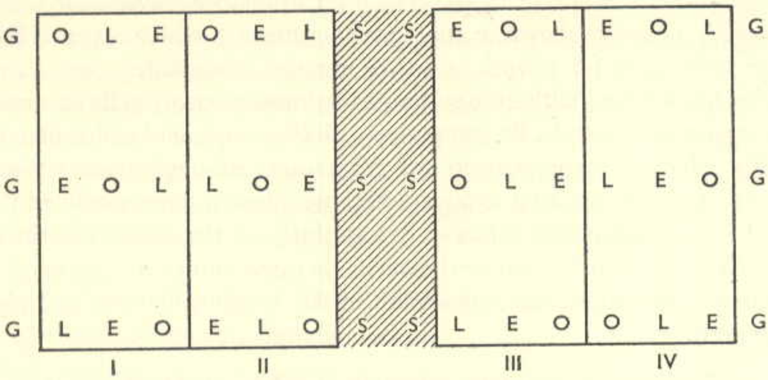

Fig. 1. A representative plot of the 1965 experiment showing the distribution of virus-infected sources (S), gooseberry guards (G) and subplot 'target' bushes. These were arranged in Latin squares (I- IV) and unsprayed (O), or sprayed with endrin (E), or lime sulphur (L).

Observations

The incidence of shoots with mite-affected foliage was recorded in mid-summer. Infested buds eventually became rounded galls which were counted annually after leaf-fall.

In the second year, virus-infected bushes were diagnosed by examining the blossom as flowering began and later by fortnightly inspections of the leaves. Such detailed recording distinguishes separate infection points (Thresh, 1965*b*, 1966*b*).

RESULTS

The spraying experiment with healthy and virus-infected bushes, in 1966

Immediately before each spray application, six shoots were collected from a representative sample of the experimental bushes in each treatment. Detailed examination and dissection under a binocular microscope revealed very few mites until the third occasion on 9 May. On the unsprayed bushes, mites were then numerous *in* the buds of reverted shoots and *on* the buds of healthy ones. Relatively few mites were found in or on the buds of sprayed bushes and all were at the apex or in the axils of the youngest leaves. The spray deposit must have been greatly diminished by weathering and by attenuation on shoots that had expanded since the previous applications. Consequently, a relatively non-toxic catchment area was available to dispersing mites and some were already established in or on the youngest buds of sprayed bushes.

Mites reaching shoot apices caused the leaves expanding later to be malformed. Affected shoots occurred exclusively on the reverted bushes and were least numerous on the bushes sprayed with endrin or endosulfan (Table 1). Affected shoots on unsprayed bushes later developed apical and axillary galls. Shoots of bushes sprayed with lime sulphur behaved similarly, whereas those sprayed with endrin or endosulfan were virtually free of galls (Table 1).

Records of mite-affected shoots underestimated the effectiveness of endrin and endosulfan. These materials differ from lime sulphur in eradicating mites from within buds (Collingwood & Dicker, 1960), even after immature leaves near the apex have been damaged. This was shown in another experiment in which reverted bushes were exposed to infestation for 1 week and then sprayed immediately, or 1, 2 or 3 weeks later. All bushes sprayed with lime sulphur developed as many galls and mite-affected shoots as unsprayed controls. By comparison, all the endrin and endosulfan treatments were highly effective in preventing gall formation, although bushes that were not sprayed until 2 or 3 weeks after exposure became infested temporarily and developed malformed leaves. Reversion virus spread to thirty of the eighty healthy bushes in the trial. Five lime-sulphur-sprayed bushes became infected compared with nine endosulfan-sprayed. Eight infections were in the unsprayed plots and eight in the endrin-sprayed.

Table 1. *The infestation of healthy and reverted bushes in the 1966 spraying experiment*

	Mite-affected shoots (%)		Total galls on 20 bushes	
Treatment	Healthy	Reverted	Healthy	Reverted
Unsprayed	0·0	65·0	125	842
Lime sulphur	0·0	26·2	57	265
Endosulfan	0·0	12·5	4	51
Endrin	0·0	12·9	1	10

Table 2. *The amount and distribution of endrin on the different organs of bushes that were sprayed and analysed immediately or ofter* 8 *and* 21 *days*

	Amount detected after:*			Percentage distribution after		
	0 days	8 days	21 days	0 days	8 days	21 days
Immature tissues						
Axillary buds	0·05	0·03	<0·01	0·1	0·1	0·1
Laminae	116·0	14·8	0·3	16·6	15·0	2·1
Petioles	42·0	5·5	0·4	2·8	2·2	0·6
Apex and stem	14·1	1·0	0·1	0·6	0·4	0·1
Mature tissues						
Axillary buds	0·10	0·06	0·04	0·2	0·6	1·0
Laminae	151·0	23·8	5·4	67·4	69·8	79·8
Petioles	37·2	8·1	3·0	8·3	8·4	10·8
Apex and stem	9·1	1·6	0·5	3·9	3·4	5·5
Total per shoot	378·1 μg	84·9 μg	39·1 μg	100%	100%	100%

* μg endrin per bud or per g fresh weight of lamina, petiole or stem. The distinction between mature and immature tissues was made at the time the samples were collected for analysis.

Acknowledgements are made to Mrs J. Lewis (née Sillibourne) for the analytical data incorporated in the table and in the text. Samples were extracted with 60—80° C petroleum ether and the endrin determined by gas chromatography involving electron capture.

The amount and distribution of endrin on sprayed bushes

The magnitude of the changes in deposits is apparent from analyses on shoots of additional bushes sprayed on 4 May. Immediately after spraying and 8 or 21 days later three shoots were dissected and the deposits on different organs were extracted and analysed separately (Table 2). Buds in the axils of the immature leaves near the apex are the only ones vulnerable to mites (Thresh, 1967), yet 80% of the initial deposit was on the mature fully expanded stem and leaf tissues. There was only 0·02–0·07 μg of endrin per immature bud, compared with 0·04–0·15 μg per mature bud.

The total amount of endrin detected had decreased by 78% and 90% after 8 and 21 days, respectively. The distribution between the different organs was little changed after 8 days, but after 21 days the proportion on the immature tissues had declined to only 3% of the total recovered. Many immature leaves had developed since the original spray and the youngest buds were virtually unprotected, with only 0·001–0·005 μg of endrin per bud, compared with 0·034–0·058 μg per mature bud.

The experiment on spread to and from sprayed bushes

The mite infestation of the virus-infected sources

The infested sources of virus carried approximately 200 galls per bush when planted, and similar numbers recurred on bushes left unsprayed throughout the following season. Endrin virtually eliminated mites and an average of only two galls was recorded per sprayed bush, compared with thirty-nine galls on bushes sprayed with lime sulphur.

Reverted bushes are particularly vulnerable to mites and those that initially were uninfested provided data on the spread of mites between plots. An average of sixteen galls per bush was recorded, with more galls on bushes in the eastern half of the experiment than in the western half, suggesting an influence of the west and south-west winds prevailing throughout the dispersal period in 1965.

Mite infestation of the main plots

There were differences in the infestation of the healthy 'trap' bushes exposed in the main plots (Table 3). Plots with virus source plants infested at the outset, and not sprayed, developed more galls than plots with infested sources that were sprayed ($P < 0.05$) and many more galls than plots with uninfested sources of virus ($P < 0.01$) (Table 3).

Table 3. *Mite infestation and virus infection in bushes exposed alongside infested and uninfested sources**

	Mite-infested:			Virus-infected:	
Virus sources in main plots	Bushes	Shoots	Buds	Bushes	Shoots
A. Uninfested—unsprayed	17·0 (8·6)	38·8 (17·6)	48·0 (21·4)	4·0 (1·9	4·9 (2·5)
B. Infested—unsprayed	21·6 (11·6)	78·6 (48·8)	114·4 (78·0)	7·9 (4·5)	14·3 (9·8)
C. Infested—sprayed lime sulphur	17·7 (10·4)	51·0 (31·6)	67·0 (35·4)	5·4 (3·2)	7·5 (4·7)
D. Infested—sprayed endrin	19·4 (10·8)	52·4 (28·2)	67·0 (41·2)	6·7 (3·7)	8·7 (5·1)
Standard error	— —	— —	10·1 (4·6)	1·0 (0·5)	2·2 (1·3)

* Means per plot of thirty-six bushes. Data in parentheses are for the eighteen bushes nearest to the sources and subject to least contamination from other plots.

In all plots with infested sources of virus that were not sprayed, galls were more numerous on nearby bushes than on peripheral bushes. This trend was less marked in sprayed plots and was reversed in plots with uninfested sources of virus. Consequently, variation was decreased by analysing only the data for the eighteen proximal bushes in each plot that were subject to the least contamination from outside. This was so great that it was impossible to determine the precise effects of spraying the sources.

However, the effectiveness of both endrin and lime sulphur in preventing spread of mites to adjacent bushes could be estimated if the 'background' infestation was similar for all treatments. On this assumption, endrin or lime sulphur decreased the spread of mites to approximately 30% of that from unsprayed bushes.

Mite infestation of the subplots

The infestation of the subplot bushes was influenced by their proximity to infested bushes and by the spray applications (Fig. 2). Invariably the greatest infestations were recorded on unsprayed bushes immediately alongside infested bushes that were unsprayed. Up to sixteen galls developed per bush and many plants had galls on several branches. Relatively isolated bushes and those alongside infested sprayed bushes developed fewer galls and many had none.

Endrin was almost completely effective in preventing the infestation of healthy bushes, even when they were immediately alongside infested bushes that were unsprayed. Few bushes developed galls and then only one on each affected shoot. Lime sulphur was much less effective and almost half the sprayed bushes developed galls (Table 4).

Table 4. *Mite infestation and virus infection in the bushes exposed in subplots that were unsprayed or sprayed with endrin or lime sulphur*

Subplots (240 bushes/treatment)	Total mite-infested:			Total virus-infected:	
	Bushes	Shoots	Buds	Bushes	Shoots*
O-Unsprayed	209	745	1071	152	267
E-Sprayed endrin	28	34	39	107	152
L-Sprayed lime sulphur	141	325	372	101	113

* Distinct points of infection

Virus infection in the main plots

The incidence of reversion virus in the main plots was related to the distribution of galls the previous winter (Table 3). Plots with unsprayed infested sources of virus contained more infected bushes than those with sprayed infested sources and many more than plots with virus sources that were not infested at the outset ($P < 0.05$). The numbers of distinct infections showed the same trend, with greater differences between treatments ($P < 0.01$) after analysing data for whole plots and for the proximal bushes only. This was because multiple infection was usual in plots with many infected bushes, but rare in those with few. Spraying decreased the spread of virus to adjacent bushes and lime sulphur was apparently more effective than endrin, but the interpretation of the results is complicated by cross-infection between plots.

Virus infection in the subplots

In all plots the number of unsprayed bushes that became infected with virus was less than the number with galls (Table 4). There was a similar relationship for bushes sprayed with lime sulphur, which decreased the number of galls and the incidence of virus-infection ($P = < 0.01$). In striking contrast, endrin was less effective than lime sulphur in preventing virus infection, despite the much greater efficiency of endrin against mites. Many bushes became infected even though they had not previously developed galls (Figs. 2, 3).

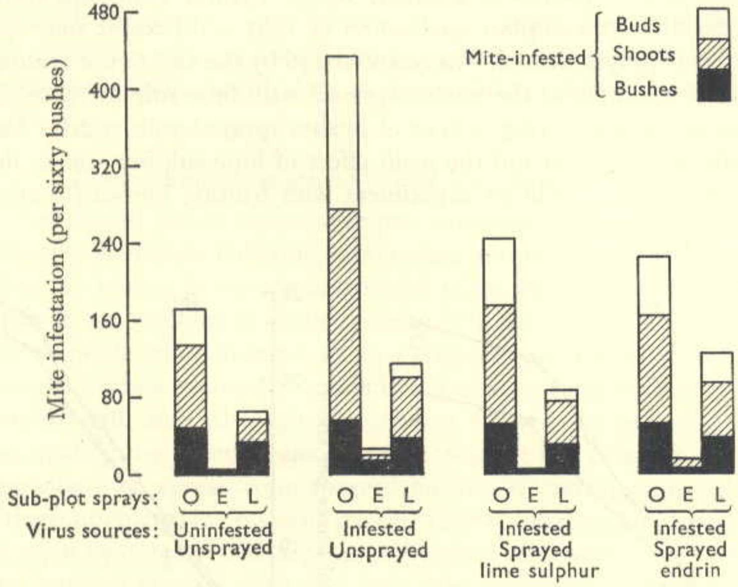

Fig. 2. The number of mite-infested bushes, shoots and buds for each combination of main and subplot treatment.

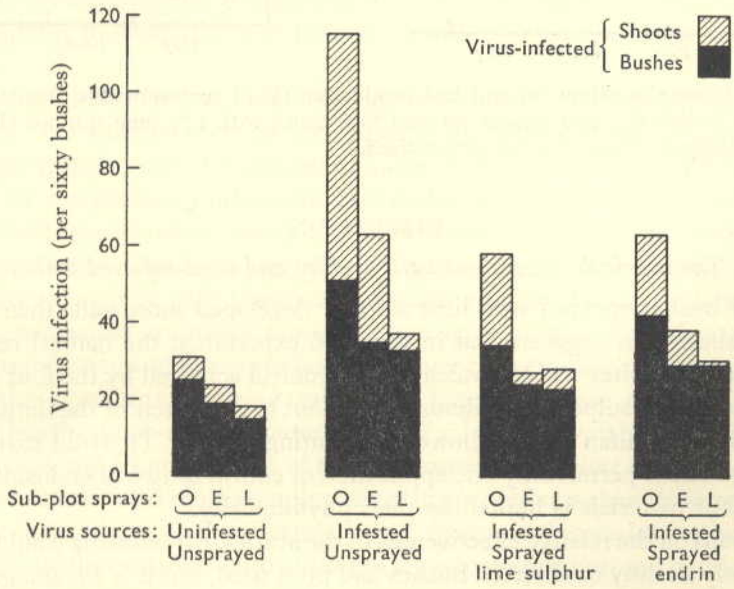

Fig. 3. The number of virus-infected bushes and distinct points of infection for each combination of main and subplot treatment.

The phytotoxicity of lime sulphur

Sulphur or lime sulphur sprays applied to blackcurrants early in the growing season frequently cause a leaf chlorosis, sometimes followed by necrosis, premature abscission and decreased growth (Kirby & Bennett, 1965). Typical symptoms developed the week after the first lime sulphur application in 1965 and became more pronounced after subsequent sprays. Growth was retarded and by the end of the season representative shoots of a sample of the bushes sprayed with lime sulphur were shorter than those of unsprayed bushes (Fig. 4*a*) or of bushes sprayed with endrin. Leaf number was little affected (Fig. 4*b*) and the main effect of lime sulphur was to decrease the mean internode length, as in an experiment with fruiting bushes (Smith & Clarke, 1967).

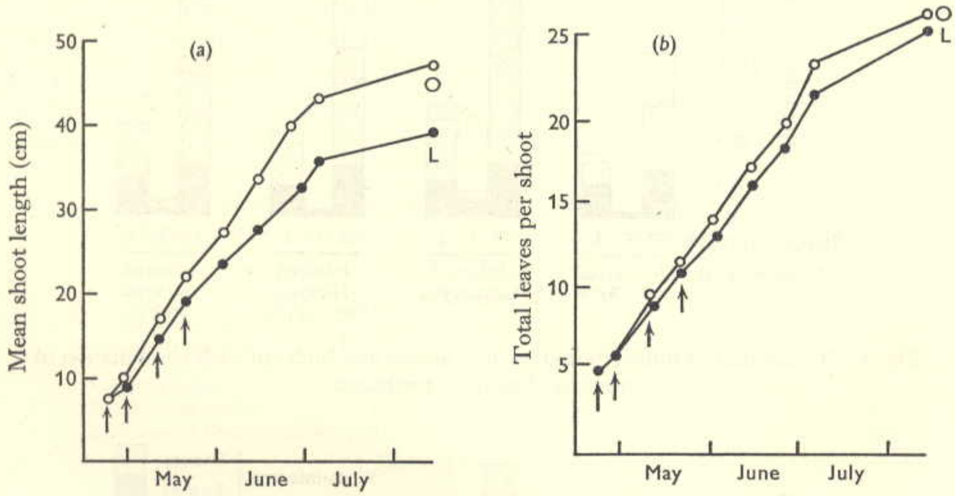

Fig. 4. Stem elongation (*a*) and leaf production (*b*) of representative shoots of unsprayed bushes (O) and bushes sprayed four times with 1 % lime sulphur (L). The arrows indicate dates of spray applications.

DISCUSSION

The acaricide experiment with healthy and virus-infected bushes

Reverted bushes sprayed with lime sulphur developed more galls than unsprayed healthy bushes. This suggests that in the 1966 experiment the natural resistance of healthy bushes to mites was equivalent to the control achieved by the four fortnightly applications of lime sulphur and almost equivalent to the effects of the limited number of endrin or endosulfan sprays allowed on fruiting bushes. To avoid toxic residues, present regulations permit only one application of endrin or two of endosulfan and the use of sulphur materials is limited by their phytotoxicity.

Conclusions on the relative effectiveness of the acaricide treatments would have been similar if only healthy or reverted bushes had been used, but it is advantageous to use both types of bushes. Reverted ones provide adequate data in years when good control is achieved or when conditions are unfavourable for mite dispersal, whereas healthy bushes are invaluable in years when there is excessive multiple infestation of reverted bushes. Healthy bushes have the additional advantage of providing data on virus spread.

Observations on both healthy and reverted bushes during the dispersal period can also provide complementary information on the way in which the different spray materials affect mites. An ability to eradicate mites from infested buds can be demonstrated readily only by using reverted bushes (Thresh, 1967). By comparison, observations on the numerous mites that occur on and around the buds of healthy bushes during the dispersal period provide data on the mortality achieved by contact poisons on plant surfaces.

The experiment on spread to and from sprayed bushes

A simple experiment design was used in previous attempts to control the spread of reversion virus by chemicals. Different sprays were compared for their effectiveness in protecting healthy bushes from infestation with mites and from infection with virus spreading from unsprayed bushes planted nearby (Thresh, 1965 *a*, *b*; Skerrett & Smith, 1967). These experiments simulated in an extreme form the situation in sprayed nurseries and plantations exposed to much infection spreading from outside sources.

Despite some limitations, the 1965 experiment showed the potential of complex designs in evaluating the effectiveness of sprays, since it was possible to consider for the first time effects on spread from sprayed bushes. However, cross-contamination was greater than anticipated in designing the experiment and prejudiced the comparison between the main treatments. The separation between plots was inadequate, although it would have sufficed in 1962, 1964 and 1966 when conditions at East Malling were relatively unfavourable for mite dispersal. Larger plots, smaller sources, additional guards and a different orientation to the prevailing wind should be considered in designing future experiments, particularly if some of the exposed bushes are reverted and so vulnerable to mites that they become heavily infested even when remote from the sources.

Sulphur and chlorinated hydrocarbon materials differ in their effects on mites. Sulphur and lime sulphur seem to act primarily as contact poisons (Jary, Austin & Pitcher, 1938) that decrease the number of mites reaching sites vulnerable to infection with virus. By comparison, both endrin and endosulfan kill relatively slowly (Smith, 1966) and their contact effect seems less marked than the ability to eradicate mites after they have invaded buds and have had an opportunity of introducing virus. Such properties are consistent with these and previous results (Thresh, 1965 *a*, *b*). Both endrin and endosulfan are much more effective against mites than they are in preventing virus infection and their excellent performance in eradicating mites from virus-infected bushes in routine screening tests gives a false impression. Clearly a material is needed that has the quick-acting, contact effect of sulphur materials and also eradicates mites from galls and from the buds of the new growth. To select such a material will require more detailed and more complex screening procedures than those used hitherto. However, a search for a safe, effective, non-phytotoxic chemical is justified because it would facilitate the control of both mites and virus spreading into and within nurseries and plantations.

Whatever the material advocated, virus-infected bushes should be removed promptly before they have become major foci of infestation. Only in these circumstances are sprays being used efficiently, and chemical control of mites should be an adjunct to roguing and not a substitute for this basic control measure.

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