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Grapevine yellow speckle disease — studies on natural spread observed in the field

by

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Grapevine yellow speckle disease — Untersuchungen über die natürliche Ausbreitung im Freiland

Zusammenfassung. — Es wurde gezeigt, daß YS unter Freilandbedingungen auf einem natürlichen — noch unaufgeklärten — Wege von kranken auf gesunde Reben übertragen wird. Die Virose breitete sich langsam, unregelmäßig und ohne Beziehung zu Jahrgang, Standort oder Sorte aus; die nächste infizierte Rebe konnte hierbei nur 0,7 m oder aber auch 5 m entfernt sein. Zu Anfang waren die gesunden Reben, die im Freiland erkrankten, zufällig über die Rebfläche verstreut und nicht notwendigerweise gehäuft beieinander.

Die in Ausbreitungsgebieten der Krankheit vorkommenden Nematoden, hauptsächlich *Tylenchorhynchus* und *Paratylenchus* spp. kamen als Vektoren kaum in Betracht. Gesunde Reben, die im Freiland oder in Töpfen in nächster Nähe infizierter Reben gepflanzt wurden, zeigten auch nach 5 Jahren keine YS-Symptome. Natürliche Wurzelvereinigungen zwischen Reben, die in Töpfen beisammen wuchsen, konnten ebenfalls nicht nachgewiesen werden.

Durch Rebscheren, die gezielt mit dem Saft infizierter grüner Triebe kontaminiert wurden, konnten YS und Blattrollkrankheit ebensowenig übertragen werden wie durch simulierten Blattkontakt. Die gelegentliche Übertragung durch Pollen oder Samen kann nicht ausgeschlossen werden.

Eine natürliche Ausbreitung von Blattroll- und Fanleafvirus wurde nicht beobachtet.

Introduction

Grapevine yellow speckle disease (YS) is a graft-transmissible disease of unknown etiology (TAYLOR and WOODHAM 1972). It is widespread in vines growing in Australia (WOODHAM *et al.* 1973), and possibly throughout the world because in recent years we have detected the disease in clones imported from California, France, Italy, Spain and Japan. In the course of our indexing programs for viruses we first observed leaf symptoms of YS on uninoculated indicator vines growing in the field in autumn 1972. This unexpected finding suggested natural spread of YS and led to the investigations reported in this paper. They comprised observations during 10 years on natural spread of YS in several widely-separated fields, and our unsuccessful attempts to determine a possible method of spread. These involved growing bait plants in the field and in pots; examinations of soil for nematode spp.; attempted transmission by secateurs experimentally "contaminated" from green shoots, or by mechanical contact of damaged shoots; and examination of seedlings and their propagules for possible transmission by pollen or seed.

Materials and methods

Field observations on spread of disease

Inspections for YS symptoms in all our sources of indicator vines and in all field experiments incorporating these vines have been made regularly during each year. The plantings concerned were on CSIRO sites at Merbein, Coomealla (10 km north of the Merbein site) and Koorlong (10 km south east of the Merbein site).

For the indexing tests young plants of indicator cultivars were graft-inoculated (hereafter called I-vines) with dormant chip-buds in spring in a glasshouse. They were planted in early summer in the field in rows where each group of 6 or 9 I-vines was interplanted with a single uninoculated vine of the corresponding indicator cultivar. These uninoculated plants are subsequently called NI-vines. Each year the annual growth on each vine was trained up a stake attached to a single-wire trellis about 1.8 m above ground.

The observations made in 1972 and 1973 of such tests (Table 1) were from three fields at Merbein, named B, P and A, where the vines had been planted 0.7—1 m apart in rows 2.5 m apart. Field B was planted in 1968 and was 5 m distant from a field of mature vines later found to be infected with YS. Field P was some 300 m distant from field B and comprised two areas each of 0.2 ha, one had previously been a vine nursery (P1) and the other had not carried vines before (P2). We used both areas in 1969 and only P2 in 1970. Field A, 300 m from field P and 100 m from field B, was planted in 1971 after removal of 30-year-old vines and was used annually thereafter.

Use of bait plants

(a) In the field. — In spring 1972 many YS-diseased vines in both areas of the 1969 planting in field P were removed from parts of 5 rows. 50 young NI-vines of either Mataro, LN 33 or Mission Seedling 1 (TAYLOR and WOODHAM 1972) were immediately planted adjacent (1 m) to existing YS-diseased vines and often they intentionally replaced a diseased vine. Many of the YS-diseased vines also carried leafroll disease (LR). All vines were pruned each winter to 6—10 nodes.

(b) In pots. — Surface soil (2—15 cm depth) and subsoil (22—37 cm), taken in spring 1972 from around NI-vines that had become YS-diseased, was mixed with 20 % and 30 % of river sand, respectively, then added to each of five 27 l pots in the ratio of 13 l to 9 l. 4 pots each contained 1 YS-diseased, 3-year-old vine that had been excavated with as big a ball of soil and roots as possible, and one young NI-vine of Mataro or Mission Seedling 1; the 5th pot contained 1 NI-vine of each indicator cultivar. We regularly inspected the plants grown in the open during 5 years. During the growing season root temperatures were kept about 21—24 °C by placing the pots into waterproof containers immersed in a water bath. The plants were kept in a shadehouse during the dormant season.

Examination of soil for nematode genera

The nematodes present in several samples of soil were identified. In spring 1972 samples were taken at 7—30 cm depth from the root zone of 7 NI-vines and 2 I-vines all of which had shown YS symptoms in field P. In winter 1975 samples to 45 cm depth from 6 NI-vines in field A were compared; 3 of these had become YS-diseased while the other 3, nearby the respective diseased vines, were still free of symptoms.

Soil from each 27 l container was sampled at budburst of the 3rd season after planting. The vines were returned to the containers and inspected for a further 2 years.

Transmission by contaminated secateurs or by mechanical contact of damaged and healthy foliage

We used 1 or 2-year-old healthy vines growing in individual pots as receptors and similar-aged vines infected with YS only or with YS and LR as donors in two transmission tests. These were done in summer of successive years in a glasshouse with temperatures between 16 and 26 °C. In experiment 1, 2 Mataro and 2 Mission Seedling 1 receptor vines were paired with YS donors, and 2 Cabernet Franc receptors with (YS + LR) donors. In experiment 2, 12 vines of Mataro or Cabernet Franc were brought into contact, half and half, with YS and (YS + LR) donors.

Infection from donor to receptor vines was attempted by using "contaminated" secateur blades and by joining shoots at freshly cut areas. For transmission by secateurs we cut the receptors through immature nodes or bases of petioles, 3–6 times in experiment 1 and 12–16 times in experiment 2; the blades were kept moistened with sap and fragmented tissue by repeatedly cutting young and mature leaves of the respective donors, and were also drawn across the cut surfaces. In 2 of the 6 donor-receptor pairs of each infection in experiment 2 the secateur blades were repeatedly dipped into a 2% tri-sodium orthophosphate solution before cutting donor and receptor tissue.

In addition, 1 or 2 shoots of each donor-receptor pair were joined at newly made cuts through nodes in experiment 1, and at areas where 2–5 cm long slivers of tissue had been freshly removed in experiment 2. These were tied to permit exchange of sap thus simulating contact of mechanically damaged shoots. In experiment 1 the receptor shoots were separated from the donor shoots after 1 week and newly made cuts were again "contaminated" by secateurs as described above. In experiment 2 half the receptors of each infection were also separated after 1 week at which time, in both experiments, sap flow had ceased and no callus was evident. The other half of the receptors were separated after 24 or 48 h when bleeding was profuse; these were "recontaminated" by secateurs as in experiment 1, and by 30–40 new cuts made on each vine, mostly into immature stems, with a "contaminated" knife.

All receptor vines remained in pots until the next spring when they were planted without previous pruning at 1.2 m spacing in a field that had not carried vines previously. They were compared for 4 (experiment 2) or 5 (experiment 1) years with NI-vines planted concurrently. All vines were pruned each winter to ground level retaining about 10 nodes and ensuring no contamination of secateurs between receptors and NI-vines. The shoots of adjacent vines did not intermingle in any growing season.

Transmission by pollen or seed

Periodic inspections of several populations of seedlings produced within the Division's breeding program (ANTCLIFF 1978) have been made to examine the possibility of spread during sexual reproduction.

In autumn 1973 we observed a seedling population produced from a Sultana infected with YS and LR as the male parent and Merlot infected with YS as the female parent. 87 of these seedlings had been grafted in their 4th year, 1971, to 40 healthy Mataro and 47 Cabernet Franc scions (indicators for YS and LR); another 29 ungrafted seedlings were inspected at the same time. The seedlings had been planted in a field previously planted to Sultana. All 70 clones of this cultivar that have been indexed contain YS (TAYLOR and WOODHAM 1972) and also LR (WOODHAM and KRAKE 1978).

In autumn 1981 propagules of 83 seedlings that had been obtained by crossing Sultana (σ) with one of 36 different cultivars (φ) were inspected. The YS status of most female parents was unknown. Half of these seedling selections had been planted in each of two fields, about 400 m apart, that had been previously planted with Sultana. There were 6–8 propagules of each seedling aged 4–8 years.

Results

Evidence for natural spread of YS

Spread of YS occurred in NI-vines used amongst closely-planted vines (0.7–1 m apart) in indexing tests (Table 1), in source plantings of indicator vines (Table 2), and in two further experiments at Koorlong (Table 2). Spread was irregular with considerable differences and inconsistencies between years, sites, and cultivars.

In the NI-vines (Table 1) symptoms first occurred in autumn 1972 on hybrid LN33 and Mataro (syn. Esparte), 2 of 4 cultivars we use to detect YS. The presence of YS in these vines was verified by the subsequent results from indexing tests. Some NI-vines first displayed YS symptoms 3 years after planting, while others showed the first signs of infection after 4 or 5 years. The seasonal differences were most striking and their cause is unknown; most new symptoms occurred in 1972 and 1973 while none were observed between 1977 and 1981 although YS-diseased vines were growing only 1 m from many NI-vines.

The plantings of indicators for source vines (Table 2) were established at widely separated sites — Merbein, Coomealla 1 (10 km north of Merbein), and Coomealla 2 (500 m from Coomealla 1). LN33 vines, derived from cuttings that were newly imported as clonal material from the University of California, Davis, were planted in 1965 at Merbein (10 vines) and at Coomealla 1 (3 vines); each planting was 3 m from vines subsequently found to be infected with YS. A further 15 LN33 vines, propagated from Co-

Table 1

Natural transmission of yellow speckle disease in indexing tests at Merbein · Denominator = no. of NI-vines observed each autumn · Numerator = no. of NI-vines showing YS in that year for the first time · Observations for 1972 and 1973 are also arranged in the respective fields planted

Die natürliche Übertragung von YS bei Indikatorversuchen in Merbein · Nenner = Anzahl der jeden Herbst ausgewerteten NI-Reben · Zähler = Anzahl der NI-Reben, die in dem betreffenden Jahr erstmals YS zeigten · Für 1972 und 1973 sind auch die Beobachtungen aus den angepflanzten Parzellen eingetragen

Year observed	Indicator cv.				Field observed and date of planting				
	Cab. Franc	Mataro	Miss. Seedl. 1	LN 33	B 1968	P1 1969	P2 1969	P2 1970	A 1971
1972	0/67	4/47	0/8	3/74	0/20	6/41	1/21	0/61	0/53
1973	1/63	3/39	1/8	13/57	4/20	8/22	1/11	5/61	0/53
1974	0/75	0/55	0/19	1/17					
1975	0/85	1/65	0/29	1/19					
1976	0/36	1/34	0/24	0/14					

Note: All observations in 1974 and thereafter were from field A. Some 104–135 NI-vines in further indexing tests were observed annually from 1977 to 1981 but none showed YS symptoms.

Table 2

Natural spread of yellow speckle disease in indicator vines planted in widely-distant fields
Die natürliche Ausbreitung von YS bei Indikatorreben aus weit voneinander entfernten Parzellen

Site	Cultivar	Planted		YS symptoms first observed		No. with symptoms to 1981
		Year	No.	Year	No.	
Merbein, Field C	LN33	1965	10	1974	2	8 ¹⁾
	Cabernet Franc	1965	8			Nil
	Mataro	1965	15			Nil
Coomealla 1	LN33	1965	3	1974	1	3
Coomealla 2	LN33	1966	15	1976	3	7
	Baco 22A	1966	12	1981	1	1
	Cabernet Franc	1970	15			Nil
	Mission Seedling 1	1970	15			Nil
Koorlong	Cabernet Franc	1972	26	1978	2	2
	Cabernet Franc	1976	8	1980	2	2
	LN33	1977	7	1980	1	1
	Mataro	1976	8			Nil

¹⁾ All 10 vines were removed in 1976.

mealla 1, were planted in 1966 at Coomealla 2, some 5 m from vines later found to contain YS and LR. Both at Merbein and Coomealla 1, LN33 vines first showed YS symptoms in autumn 1974, and 8 and all 3 vines, respectively, were diseased by 1976; but Coomealla 2 vines showed no symptoms until 1976 and newly diseased vines were again found in 1977, 1978, 1979 and 1981. At each site the diseased LN33 were randomly scattered within the row. Of the other indicator cultivars that adjoined LN33 in Coomealla 2, only 1 of 12 Baco 22A vines showed symptoms, 15 years after planting. Furthermore, Cabernet Franc and Mataro, intermixed with other cultivars containing YS at Merbein, have remained healthy for 16 years after planting.

In one of the two experiments planted at Koorlong (10 km south-east of Merbein) 26 healthy Cabernet Franc and 52 vines of the same clone deliberately infected with YS plus LR were planted 1972 in an area not previously planted to vines. Each of the healthy vines was growing 2.5 m from adjacent infected vines that have expressed symptoms of YS and LR each year. 2 of the 26 healthy vines, some 35 m apart, first showed YS symptoms in 1978, but none have become diseased since.

The second experiment, established in the same area at Koorlong in 1976 and 1977, comprised healthy Cabernet Franc, Mataro and LN33 vines propagated from sources that had never shown YS symptoms, and corresponding vines experimentally infected with YS or LR or fanleaf virus. YS symptoms first appeared in 1980 on 1 of 7 healthy LN33 in the 3rd year and on 2 of 8 healthy Cabernet vines in the 4th year. The healthy plants which became diseased were 5 m within the row from experimentally-infected vines that showed YS symptoms, and were 10–17 m downwind from older vines with YS.

Bait vines in the field and in pots

We observed 30 of the bait vines spread over four rows in field P for 3–5 years and the other 20 for 1 year. To guard against excessive shading of the bait plants which may

have prevented foliar expression of YS, the aboveground portions of older YS-diseased vines were removed when necessary; the root systems and thus the populations of soil fauna were not disturbed. After 3 years only the bait vines remained; they all grew well each season. None of these bait plants showed YS symptoms throughout the periods of observation of up to 5 years. In contrast, another 9 NI-vines of the 1969 planting first expressed YS symptoms in 1973, in addition to those that had become diseased earlier. Also, YS symptoms appeared annually on all infected NI-vines and some remaining I-vines. Inspections of source vines of each indicator revealed no YS symptoms in 1972 or 1973.

All vines planted in the 5 pots grew satisfactorily each season. YS symptoms occurred on diseased vines in 3 of the 5 years but never on any plant serving as bait. Likewise, cuttings taken after 3 years from each bait vine and grown in pots in the open in threefold replicates failed to show symptoms. This confirmed the lack of transmission to these bait plants.

Nematode genera

In the field P samples, *Tylenchorhynchus* and *Paratylenchus* spp. were detected most commonly with *Pratylenchus minyus* usually present in small numbers; a very few of *Criconemoides* and *Meloidogyne* spp. were found in two samples.

These and also *Tylenchulus* spp. were present in field A. There was no apparent correlation between the presence of YS symptoms and nematode spp.

The soils of each 27 l container carried mainly *Paratylenchus* spp. after 3 years. When inspected at budburst all plants had a healthy vigorous root system with new root initials. There was no evidence of natural grafting of roots in the surface portion of the root mass that was inspected after 3 and 5 years.

Transmission by secateurs or by mechanical contact of damaged foliage

Both attempts to transmit YS or LR mechanically by the methods described were unsuccessful. None of the receptor vines showed symptoms at any stage.

Pollen or seed transmission

Inspection in 1973. — Of the 87 seedlings grafted to indicator scions, 1 Cabernet Franc scion showed YS symptoms and all were free of LR symptoms. Also, all the 29 ungrafted seedlings appeared healthy.

Inspection in 1981. — In one of the two fields, only 1 of 7 propagules of 1 seedling showed YS symptoms. The seedling itself, which had been moved into the same plot, was healthy which suggests that the one propagule had become infected while in this field. In the other field all propagules of 1 among 43 populations expressed YS. The seedling itself (seedling A), planted in 1966 in succession to Sultanas elsewhere, also showed YS when examined in 1981. Seedling A was only 1 of 9 seedlings selected from the same cross, which involved a female parent of unknown YS status, that expressed YS. Furthermore, 3 seedlings obtained from a cross with a YS-infected female parent failed to show YS. Thus, one must assume that seedling A became infected in the field before cuttings were propagated.

Carina, a selection released by this Division (ANTCLIFF 1975), is the only other known instance of a YS-diseased seedling at Merbein. The original Carina was planted 1965 in the same area as the diseased seedling A. The original seedling and all propagules at Merbein were expressing YS when closely inspected for the first time in

autumn 1979. Also, YS symptoms on Carina propagules planted at Irymple were recorded by SHANMUGANATHAN and FLETCHER (1980). Thus, most probably all Carina vines will contain YS.

Discussion

These data demonstrate that natural transmission of YS in the field occurred, but showed no regular pattern in various cultivars, in different years and on different sites. Although we have no experimental evidence we think YS has little effect on vine performance; however, any natural spread could be important particularly in nursery or source-vine areas or in foundation plantings. Natural spread has been evident in closely planted vines, < 1 m apart, suggesting underground spread perhaps by a soil vector or by natural root grafts. In such plantings shoots intermingle, particularly those of LN33 which grows most vigorously, and there is considerable contact of foliage which could also provide a possible means of spread. However, we failed to transmit disease by simulated contact of damaged shoots, did not observe root grafts and could not find nematode spp. that may act as vectors.

On the other hand, spread has occurred also when healthy and infected vines were 2.5 and 5 m apart with shoots well-separated, and perhaps even when 10—17 m apart. In addition, the NI-vines that became diseased were often randomly scattered within the field and not in closely-clustered areas. These two findings suggest a possible aerial vector.

In our annual indexing tests from spring 1973 (observed in autumn 1974 to 1976) we have taken precautions to avoid possible cross infections between material from clones of unknown health status under test (candidates) during grafting, and between I- and NI-vines before planting in the field. Also, the dormant NI-vines were all pruned separately from the I-vines. A possible relationship between increased hygiene and reduced spread (Table 1) must be questioned because we were unable to transmit the disease on contaminated tools in summer, and SHANMUGANATHAN and FLETCHER (1980) failed to induce spread by pruning implements in winter and in summer. However, it is still possible but considered unlikely that contamination between candidate material during grafting may be a method of spread.

Some viroid-caused diseases are transmitted by human contamination and by foliage contact. Both citrus exocortis (GARNSEY and WHIDDEN 1973) and chrysanthemum stunt (HOLLINGS and STONE 1973) were spread with contaminated tools; this was further increased in the case of chrysanthemum stunt by dipping the tools into 2 % tri-sodium orthophosphate. Chrysanthemum stunt was also transmitted by handling plants and by foliage contact.

YS symptoms on some source-vines of LN33, originally imported from California, were first noted in 1974, 10 years after planting in the field in Australia. It seems more likely that infection occurred after planting in the field rather than through the importation of an infected cutting. Therefore, we think it improbable that LN33 cuttings propagated for indicators between 1968 and 1971 would have been infected with YS. All subsequent LN33 indicators were propagated from healthy vines at Merbein or Coomealla 2. Also, the clones used as source vines of the other three indicators Cabernet Franc, Mataro and Mission Seedling 1 have never shown YS symptoms. Thus, the probability of the source vines having a latent infection or that an occasional cutting in some way carries a very attenuated strain which requires variable periods of incubation before expression is very small.

Soil samples from two fields contained nematode spp. that have not been reported to transmit viruses between grapevines. They are mainly migratory types commonly occurring in most soils.

Prolonged delays of up to 3 years in the appearance of foliar symptoms of virus on previously healthy plants grown in soils infested with viruliferous nematodes have been reported for fanleaf virus in grapevines (HEWITT *et al.* 1958) and for arabis mosaic virus in hops (VALDEZ *et al.* 1974). Our bait plants for YS, of unknown etiology, were inspected for up to 5 years. This should have been a sufficiently long period to effect any transmission by underground means and for the expression of leaf symptoms.

Although natural root grafts have been reported in forest trees (GRAHAM and BORMANN 1966) and in apple trees (DHINGRA 1972) we have not seen any similar evidence in grapevines grown in small or large containers or in the field, and a comprehensive survey of literature since 1960 has revealed no reports of this phenomenon in *Vitis* spp.

Our results do not conclusively exclude the possibility of transmission through pollen or seed as the low incidence of transmission is within the limits of such spread. However, assuming that all seedlings inspected were capable of exhibiting YS and LR, the overall observations and extreme variability of spread suggest that YS and LR are rarely if ever transmitted by these means.

These findings present additional information to that of SHANMUGANATHAN and FLETCHER (1980) who, since this work was started, have reported that YS appears to spread in the field at Irymple, some 11 km from Koorlong. Whichever way spread occurs, any vector is obviously inefficient or it irregularly inhabits vineyards and/or attacks vines. Perhaps YS has an alternate host which appears and/or is fed on irregularly. There is also the possibility of transmission by soil fungal pathogens or through infected exudates in the soil. Nevertheless, we consider that valid indexing for YS would never or at worst rarely be endangered if healthy indicator material and obvious hygiene measures were taken.

Throughout this work we found no evidence of natural spread of LR or fanleaf virus. This, however, has been reported for corky bark and stem pitting diseases in Mexican vineyards, but the method(s) of transmission is unknown (TELIZ *et al.* 1980).

Summary

Evidence of a low incidence of natural transmission of YS between vines in field situations by an undetermined method is presented. Spread to healthy vines occurred irregularly and erratically between various cultivars on different sites and in different years; it occurred where an infected vine was growing 0.7 and up to 5 m distant. Initially healthy vines which became diseased in the field were randomly scattered and not necessarily within clustered areas.

The nematodes present in areas of spread, mainly *Tylenchorhynchus* and *Paratylenchus* spp., were unlikely to be the vector. Healthy vines planted as bait next to infected vines in the field or in containers did not show symptoms after 5 years. There was no indication of natural root grafts on vines growing in containers.

Transmission of YS and of leafroll did not occur by scateurs deliberately contaminated with sap from infected green shoots or by simulated foliage contact. Rare transmission through pollen or seed still remains a possibility.

Natural spread of leafroll and of fanleaf virus was not detected.

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