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dei Virus e delle Virosi della Vite

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ISTITUTO DI PATOLOGIA VEGETALE, UNIVERSITÀ, MILANO

VIROSI E SELEZIONE CLONALE DELLA VITE ALL'ANNO 1973: INTRODUZIONE AL CONVEGNO

E. BALDACCI

Con il presente Convegno si esaurisce il programma degli incontri previsti dal nostro Gruppo (I.C.V.G.) ed iniziati, come ben sapete, a Nyon (Svizzera) nel 1964, proseguiti nel 1965 a Davis (California), nel 1967 a Bernkastel (Germania) e nel 1970 a Colmar (Francia). Oggi, nel 1973, mentre concludiamo in Italia il primo decennio di un lavoro comune porgo l'augurio per tutti noi di un altro decennio di proficue riunioni a bilancio delle ricerche svolte singolarmente.

All'attivo del lavoro svolto dal gruppo stanno alcune altre iniziative fra le quali emergono i due fascicoli della rassegna bibliografica delle virosi della vite. La rassegna comprende nel primo fascicolo la bibliografia dalle origini al 1965 (Caudwell, 1965) e nel secondo quella dal 1965 al 1970 (Hewitt *et al.*, 1972). Ricordo ancora gli elenchi e gli scambi di materiale viticolo selezionato e di antisieri. Ma frutto maggiore è certamente questa comunione di intenti e di ricerca perseguita in tanti anni, questo porre a base della indagine una terminologia ad una metodologia accettate liberamente ed una critica comune dei risultati.

Quando ci riunimmo per la prima volta nel 1964 alcuni chiarimenti fondamentali erano stati già acquisiti (Belli, 1969). Era caduto l'equivoco provocato da una terminologia univoca, quello della « degenerazione infettiva ». Sapevamo per converso che molte erano le malattie da virus nella vite, fra loro distinte e distinguibili. Il fan-leaf, l'yellow mosaic, il vein-banding ed il leaf-roll furono così riconosciute nei singoli paesi viticoli ed ebbero il battesimo nominale nella lingua di ogni paese. L'asteroid mosaic, la flavescence dorée, la Pierce's disease ed il corky bark risultarono territorialmente delimitate con proprie fisionomie patogeniche. Il numero dei virologi della vite si è incrementato negli anni delle nostre riunioni e notevolmente aumentato è il lavoro di ricerca. Viene fatto giustamente notare dai compilatori della rassegna bibliografica che nel periodo di cinque anni, dal 1965 al 1970, sono stati raccolti 332 riferimenti contro i 1053 dei 50 anni precedenti (Hewitt *et al.*, 1972). Se è pur vero che ciò si deve per una parte allo sviluppo di quella nuova scienza che è la virologia vegetale, si può riconoscere senza ombra di ambizione che per una parte vi ha concorso il richiamo esercitato dal Gruppo e dalla sua brillante attività.

Non starò ad elencare il lavoro svolto in questi anni perchè vi è ben noto ed è stato già oggetto di rassegna. Credo più utile discutere un poco confidenzialmente di ciò che non è stato raggiunto o acquisito. Non conosciamo l'eziologia del leaf-roll ma vi è di più. Le nostre conoscenze su questa malattia sono limitate ed unico elemento positivo è la possibilità di riconoscerla attraverso la trasmissione in piante di vite sensibili. Ben pochi studi sono stati svolti in argomento: la citata rassegna bibliografica elenca solo 20 lavori dal 1965 al 1970! Una situazione analoga si ha per il Corky-bark e per il legno riccio; ma mentre queste malattie hanno diffusione limitata, il leaf-roll è riconosciuto presente in tutte le aree viticole!

Qualche altra malattia è stata riconosciuta ma solo attraverso piante test mentre un certo numero di virus noti e di virus latenti sono stati riscontrati su vite, ma non è stabilito se si tratta di « contaminazioni in campo » o se essi concorrano con altri in determinate occasioni a provocare anomali quadri morbosi. Anche le ricerche di microscopia elettronica non hanno avuto ampio respiro e ciò è da porre forse in connessione con la carente ricerca ezio-

logica. Una micoplasmosi è considerata la flavescence dorée, sebbene la dimostrazione della patogenicità del micoplasma non si sia potuta ancora effettuare. Osservo che tutto l'intero gruppo di malattie attribuite a questi nuovi agenti ha bisogno di una attenta analisi sperimentale, ai fini della dimostrazione eziologica. Il fatto saliente, a mio parere. è che la tecnica di lavoro e di studio per queste malattie si discosta in parte da quella delle virosi per avvicinarsi a quella delle malattie batteriche e fungine, richiedendo l'isolamento colturale del patogeno su sustrati artificiali. E' necessario allora che noi poniamo attenzione a introdurre concetti e tecniche nuove nel nostro programma di lavoro. Non pochi studiosi ritengono che relazioni fra micoplasmosi e malattie non ancora definite siano possibili, ad esempio Bovey (1970) avvicina il Corky-bark alla flavescence.

Le ricerche sulla epidemiologia, sui nematodi e sugli insetti vettori, sulla selezione e sulla termoterapia, sulla produzione di piante virus-esenti hanno avuto notevole impulso per effetto delle conoscenze acquisite superando anche le stesse difficoltà eziologiche soprarícordate. Ciò ha richiamato l'attenzione dei nostri colleghi studiosi di problemi di viticoltura. L'Office Int. de la vigne et du vin (OIV) ha dedicato alcune sedute al problema; nel 1966 ha trattato in apposita seduta la produzione di legno (Baldacci, 1966) e ripete l'argomento nella sessione del corrente anno a Madrid. Una seduta congiunta fra noi e l'OIV fu svolta, come sapete, a Montpellier nel 1970, e fu dedicata alle malattie da virus e ai metodi di prevenzione e di lotta. Ricordo anche un simposio internazionale sulla selezione clonale promosso a Trier per opera del Zentralstelle für Klonenselektion der Landes-Lehr-und Versuchsanstalt für Weinbau, Gartenbau u. Landwirtschaft a cura del Dr. Faas e del Dr. Schöffling nel 1971 e al quale molti di noi hanno partecipato. In questa occasione furono recepite questioni e tecniche della selezione clonale postulate dal nostro lavoro, insieme alla selezione per la resistenza a malattie crittogamiche. L'iniziativa sembra prometterne altre se si tiene conto che il Simposio si è annunciato primo in argomento.

Probabilmente iniziative simili si sono prese in altri paesi e vorrete perdonarmi se non ne sono al corrente. Ciò che mi preme mettere in evidenza è che la selezione clonale, così come risulta necessaria dalle conoscenze sulle virosi, si è imposta in ogni ambiente viticolo. In particolare la selezione morfologica o visuale ha

dato frutti manifesti e solleciti sicchè essa è stata riconosciuta ovunque la base necessaria di lavoro ed « indispensabile in ogni caso » (Hewitt *et al.*, 1972). Certamente essa deve essere integrata da un successivo lavoro che può essere lungo e faticoso per talune « varietà » o « razze » di vite e meno per altre. Talvolta, in Italia almeno, si è bizzantineggiato se la selezione sanitaria deve precedere o seguire quella detta « varietale » mirante a fissare talune caratteristiche colturali e produttive; ma i più oggi sono convinti che occorre procedere insieme e che non vi è una questione di precedenze concettuali.

La nostra metodica di selezione porta inevitabilmente alla ampelografia di « tipo » e in particolare di « olotipo » anzichè a quella più tradizionale di una entità media ideale risultante dall'esame delle fluttuazioni di un numero maggiore o minore di campioni. Ciò implica una inevitabile revisione del lavoro ampelografico finora svolto. Per quanto gravosa essa sia, occorrerà effettuarla. (Ciferri, 1951).

L'eliminazione delle virosi si è confermata un mezzo di miglioramento della coltivazione e della produzione sicchè nelle disposizioni di ogni paese aderente al Mercato Comune Europeo sono state introdotte obligatoriamente clausole relative a queste operazioni selettive sanitarie. Per quanto riguarda l'Italia le norme stabilite dal nostro legislatore non mi hanno del tutto soddisfatto, come ho reso di pubblico dominio (Baldacci, 1971). Spero vivamente che alcune nostre realizzazioni che avrete modo di visitare, effettuate con l'ausilio di Enti nazionali come il Consiglio Naz. delle Ricerche e il Ministero della Agricoltura, permettano di codificare meglio in futuro il lavoro selettivo che deve essere svolto per la produzione di materiale da riproduzione. Certamente in un paese come il nostro dove la vite è coltivata in ogni regione e dove vi è una numerosa falange di « razze », il miglioramento della vite si presenta lungo e da suddividere per ambienti e zone tipiche di coltura.

Ma l'imponenza del lavoro da svolgere non ci arresterà certamente nella nostra ricerca. Con l'augurio di un buon successo anche per questo V° nostro Convegno, Vi dò il benvenuto nel nostro paese che prende nome di Enotria o terra del vino, da un certo Enotrio sbarcato molto tempo indietro nel golfo di Taranto da dove iniziò la coltivazione della vite.

Virus diseases and clonal selection of grapevines in the year 1973: introduction to the meeting

by

E. Baldacci

The present conference concludes the programme of meetings scheduled for our Group (I.C.V.G.). The first, as is well known, was held in Nyon (Switzerland) in 1964, then in 1965 in Davis (Calif.), in 1967 in Bernkastel (Germany) and in 1970 in Colmar (France). To-day, in Italy in 1973, as we draw towards the end of the first decade of collaboration I wish us all another ten years of profitable meetings on our research carried out individually.

On the part of the active work of the Group, there are some initiatives among which emerge two publications dealing with the bibliography of virus diseases of grapevines The first covers the literature from its origin up to 1965 (Caudwell, 1965) and the second that from 1965 to 1970 (Hewitt *et al.*, 1972). I must also mention the lists and the exchange of selected grapevine material and antisera. But the greatest benefit is most definitely this communion of interest and research carried out over many years, this giving as a basis to the investigations a terminology, a freely accepted methodology and a common criticism of the results.

When we met for the first time in 1964 a few fundamentals had already been acquired (Belli, 1969). The ambiguity caused by a univocal terminology, that of « infective degeneration » had been overcome. Conversely, we knew that many distinct and distinguishable virus disease exist in the grapevine. Fan-leaf, yellow mosaic, vein-banding and leaf-roll were recognized in each vine growing country and had been given a name in the language of each. Asteroid mosaic, flavescence dorée, Pierce's disease and corky bark were delimited territorially with their own pathogenic physiognomy.

The number of grapevine virologists enlarged during the years of our union and the research work increased. It was indeed noted by the compilors of the bibliographic survey that during the five years from 1965-1970, 332 references were collected compared with 1053 for the previous fifty years (Hewitt *et al.*, 1972). If this is partly due to the development of that new science, plant virology, it must be recognized, without false pride, that it is also due to the work of the Group and its brilliant activity.

I will not wait now to list the work carried out during these years as it is well known and has already been summarized. I believe that it is more useful to discuss together that which has not yet been achieved. De do not yet understand the aetiology of leaf-roll. Our knowledge of this disease is limited and the only positive element is the possibility of recognizing it by means of transmission trials into sensitive grapevine plants. Very few studies have been carried out in this subject, in fact, only twenty are listed in the summary of the work from 1965-1970. A similar situation exists for

corky-bark and for « legno riccio » (rugose wood); but while these diseases are limited in extent, leaf-roll is present in every grapevine growing area.

Some other diseases were recognized only by transmission through test plants while a certain number of known viruses and latent viruses have been found in grapevines. However it has not been established whether these are « field contaminants » or if they concur with others, on certain occasions, to produce anomalies. Even research using electron microscopy did not have an ample enough development, perhaps due to a limited aetiological research.

Flavescence dorée is considered to be caused by a mycoplasma, although the pathogenicity of the latter has not yet been demonstrated. I must add that the whole group of diseases attributed to these agents needs experimental analysis to show their aetiology. It seems to me an important fact that the technique used for working on and studying these diseases is to a certain extent distinct from that used for virus diseases and more similar to that for bacterial and fungal diseases since the pathogen is isolated and cultivated on artificial substrata. We need to introduce new ideas and techniques in our programme of work. According to not a few researchers relationships between mycoplasma and not yet defined diseases are possible; for example Bovey (1970) indicated a connection between corky-bark and flavescence.

The research on epidemiology, on nematodes and on insect vectors, on selection and on heat treatment, on the production of virus free plants was notably increased on account of the knowledge acquired even in spite of the aetiological difficulties listed above. That attracted the attention of our colleagues working on viticulture. The Office Int. de la Vigne et du Vin (OIV) devoted some sessions to the problem: in 1966 the production of wood (Baldacci, 1966) was the subject of a discussion and again this year in Madrid. A joint meeting between ourselves and the OIV took place, as you know, in Montpellier in 1970, on virus diseases and the methods for their prevention and treatment. I must also mention an international symposium on the selection of clones which took place in Trier and was organized by the Zentralstelle für klonenselektion der Landes-Lehr-und Versuchsanstalt für Weinbau, Gartenbau und Landwirtschaft (Dr. Faas and Dr. Schöffling) in 1971, at which many of us participated. On that occasion several points on techniques for clonal selection, proposed by our Group, and also on selection for resistance to cryptogamic diseases, were accepted. This seems promising if one remembers that it was only the first on the subject. Possibly similar initiatives have been taken in other countries and I hope you will forgive me for not being aware of them. The point that I want to make is that clonal selection, which, from our knowledge of virus diseases, is so necessary, has been accepted in every grapevine cultural area. In particular the morphological or visual selection has yielded clear and rapid results and so has been recognized everywhere as a necessary working basis and « indispensable in every case » (Hewitt *et al.*, 1972). Certainly it has to be integrated with a successive work which, for some varieties or races of grapevines, could be long and difficult, while for others less so. Sometimes, in Italy at least, hair splitting arguements take place on whether the selection of healthy material must precede or follow that called « varietal », which tends to fix some cultural and productive characteristics; but to-day the majority are convinced

that it is necessary to proceed simultaneously with both and that there is no question of conceptual precedence.

Our methods of selection inevitably lead to the ampelography of « type » and in particular of « holotype » rather than to the more traditional average ideal entity which results from an examination of variations of a greater or lesser number of samples. That would mean a re-examination of the ampelographic work carried out so far. However irksome this may be the task must be done (Ciferri, 1951).

The elimination of virus diseases has been confirmed as a successful means of better cultivation and production so that by prevision of the law of each country belonging to the European Common Market obligatory conditions were introduced relating to the selection of healthy plants. As regards Italy, in my opinion, the norms layed down are not entirely satisfactory as I have already stated in public (Baldacci, 1971).

I sincerely hope that some of our initiatives, which you will be able to visit, and which are supported by the National Council of Research and the Ministry of Agriculture, will lead in the future to better selection work necessary for the production of propagation material. Certainly in a country such as ours, where grapevines are cultivated in every region and where there are numerous « races », improvement of the grapevine is a long work and needs to be divided up depending on the environment and on the zone typical of the cultivation.

But the magnitude of the work to be done will certainly not deter us from our research. With best wishes for success and for our 5th meeting I welcome you to this country which gets its name Enotria or country of the wine from a certain Enotrio who disembarked, a long time ago in the gulf of Taranto, from where he began cultivating grapevines.

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SESSIONE I

NUOVE MALATTIE E NUOVI DATI SU MALATTIE GIÀ CONOSCIUTE

New diseases and new data on known diseases

(*chairman*: R. Bovey)

* CSIRO DIVISION OF HORTICULTURAL RESEARCH, MERBEIN, VICTORIA
+ VICTORIA PLANT RESEARCH INSTITUTE, DEPARTMENT OF AGRICULTURE, BURNLEY

GRAPEVINE VIRUS AND VIRUS-LIKE DISEASES IN SOUTHERN AUSTRALIA

R.C. WOODHAM *, R.H. TAYLOR +, and L. R. KRAKE *

INTRODUCTION

Our virus indexing programme has included numerous clones of Sultana (syn. Thompson Seedless, Sultanina) of varying performance, selected clones of the main wine and table cultivars, some rootstocks likely to be used in Australia, and imported grapevines of unknown virus status. Because we have found previously unreported infections in several imports we now index all imported clones for grapevine yellow speckle (GYS) (Taylor and Woodham, 1972) and leafroll. It is of interest that Englebrecht (priv. comm.) has recently noted GYS symptoms on Mission in South Africa.

Knowledge of the virus status enables clones with minimum virus load to be used by industry in the absence of data on vine performance. We consider that the best available material at any given time, independent of virus status, should be released to growers particularly in areas where rootstocks are not used and where vectors are absent.

RESULTS AND DISCUSSION

The virus and virus-like diseases detected in grapevines growing in the Murray Valley and other Victorian districts are leafroll, GYS, fleck (Hewitt *et al.*, 1970), the three strains of fanleaf virus (GFV), and enation. Many infected grapevines contain multiple graft-transmissible diseases. Only leafroll and GYS are prevalent, often occurring in combination, and thus we are more fortunate than some other countries. Corky bark has been detected only in one non-commercial cultivar.

LEAFROLL is the most important disease and occurs in all tested Sultana, Currant and Waltham Cross (syn. Regina, Dattier). It is less common in other cultivars but is frequently detected in Cabernet Sauvignon, Grenache and Shiraz all of which have sometimes been grafted in Australia to other vinifera cultivars such as Currant or Waltham Cross. Leafroll and GYS appear to occur always together in Sultana and Waltham Cross, and reworking probably explains the prevalence of this combination in other cultivars.

Although leafroll can often be detected by observation of leaf symptoms in autumn, strains which can be detected only by indexing are present in several cultivars, e.g., in clones of Sultana, Waltham Cross, Shiraz. Nevertheless, by observing symptoms, the efficient grower or nurseryman can avoid propagating from at least the virulent strains of leafroll and this practice is recommended.

Numerous leafroll infected sources have been indexed on the three indicators Mission, Baco 22A, LN-33. Only 10% have reacted on Baco 22A, these were American rootstocks or vinifera clones that probably have been grafted on rootstocks. Mission, considered the best indicator in California, has proved inferior to LN-33 in our conditions. For example, Mission, some even after 3 years, detected only 22 of the 49 Cultana clones which gave a positive or suspect redleaf reaction on LN-33; the sources that reacted only on LN-33 were symptomless. No Sultana clone has reacted on Baco 22A. On the other hand two clones of different cultivars have reacted on Mission but not on LN-33 after 3 years.

These findings suggest that different strains of leafroll exist. Consequently several vinifera cultivars including Cabernet Franc,

Cabernet Sauvignon, Pinot Noir, Shiraz, Mataro, Merlot, Peverella are being tested for their sensitivity and reliability in detecting various strains of leafroll. Cabernet Franc seems the most promising in our environment and is being further tested using candidates which have given negative or suspect reactions on LN-33 or Mission. Indicators are being kept for longer than the usual 2-year period to check if symptom expression for some strains is merely delayed in some indicators. The characterisation of strains, based on their reactions on different indicators, may have a practical value because a current field trial to study the effect of inoculating a Sultana selection with other Sultana leafroll sources indicates that some strains depress yield more than others.

GYS occurs in all Sultana and Waltham Cross tested, in clones of many other cultivars including rootstocks, in Mission and numerous other clones imported from California (Taylor and Woodham, 1972). The economic importance of GYS is not known.

FLECK is comparatively rare in tested candidates. It occurs in some vinifera cultivars and rootstocks and always in combination with one or more of the other diseases, but has not been detected in Sultana. Fleck is reliably detected on *Vitis rupestris* cv. St. George (St. George) using chip-bud or green grafting techniques; the indicators after grafting are grown in a glasshouse or shadehouse.

GFV is prevalent only in the small district at Rutherglen where rootstocks are used. The nematode vector, *Xiphinema index*, is widespread here but has not been found in other districts. Generally the virus is latent and can be detected only by indexing. The virus incidence in this district is being gradually decreased by planting selected material on new land or land that has been free of grapevines for over twenty years.

In other districts GFV has been found only rarely. Of some 60 Sultana clones indexed only three had the veinbanding strain of GFV. This GFV strain is readily detected by mechanical inoculation to *Chenopodium quinoa* (Taylor and Woodham, 1970) but not to *C. amaranticolor*. It is more difficult to detect on St. George than the other GFV strains infecting our grapevines and, contrary to the findings in California, is not reliably detected on St. George

growing in the field in the second spring after grafting. In our conditions of high light intensity, high temperature and low humidity, the leaves on field-grown St. George fold upwards and tightly inwards which necessitates manual opening to permit reading. This difficulty does not occur in a glasshouse or shadehouse. Both veinbanding and GYS diseases are latent when inoculated to Pinot Noir and Shiraz.

ENATION was most recently observed in three Sultana clones in the autumn of one season and spring (1970) of the following season. The symptoms occurred usually on shoots towards the end of the cane and 12 shoots per vine was the highest number affected; this may be a relatively mild expression. Shoots with and without symptoms were tagged on two vines in spring 1970. Studies have been started to observe if this disease is carried in cuttings, or is graft-transmissible because there is controversy concerning graft-transmission. Furthermore, workers have consistently indexed GFV in grapevines showing enation (Granitti *et al.*, 1965); however our Sultana clones with enation are free of GFV. In spring 1971 and 1972 both the source and propagule grapevines showed no enation symptoms which further indicates the complex nature of this disease.

CONCLUSION

Our indexing results support the hypothesis that many cultivars imported from the old world before rootstocks were used for phylloxera were relatively free of virus diseases, the main exceptions being Sultana, Currant, Waltham Cross, and that subsequent spread was due mainly to indiscriminate propagation from grapevines infected before importation or by grafting on infected rootstocks or other cultivars.

However plantings of cultivars which have never been propagated by grafting still exist and their low incidence of virus has facilitated selection. Some high-yielding selections carry virus, for example all Sultana selections are infected with latent leafroll and GYS. Whether or not this complex affects performance is as yet unknown. Attempts to eliminate either member of the complex by heat treatment for periods up to 11 months have failed.

SUMMARY

Fanleaf virus strains; also leafroll, grapevine yellow specle, fleck, and enation diseases have been detected in grapevines growing in southern Australia. Leafroll and yellow speckle diseases are widespread whereas the others are comparatively rare. Most infected grapevines have more than one disease, the common combination is leafroll and yellow speckle.

Enation occurs in Sultana clones which are free of fanleaf virus but infected with leafroll and yellow speckle.

Indexing results indicate that different strains of leafroll exist. Characterisation of these strains, based on their reactions on different grapevine indicators, could be of value because some strains may not be economically important. Latent strains of leafroll and yellow speckle appear difficult to eliminate by heat therapy.

RIASSUNTO

Malattie da virus e virus-simili della vite nel sud dell'Australia

L'arricciamento (GFV), l'accartocciamento fogliare (GLRV), la malattia delle enazioni e quelle denominate rispettivamente « Grape Yellow Speckle » (GYS) e « Fleck » (GFlkV) sono state riscontrate in viti coltivate nel sud dell'Australia. GLRV e GYS sono molto diffuse, mentre le altre malattie sono relativamente rare. Nella maggior parte delle viti infette è facile trovare in combinazione GLRV e GYS.

La malattia delle enazioni è stata riscontrata in cloni di Sultanina esenti da arricciamento ma infetti da GLRV e GYS.

I risultati dei saggi su viti indicatrici hanno permesso di stabilire che esistono diversi ceppi di GLRV. La caratterizzazione di questi ceppi, mediante l'impiego di differenti viti indicatrici, potrebbe essere interessante in quanto alcuni ceppi sembrano non essere importanti economicamente. I ceppi latenti di GLRV e di GYS risultano difficilmente eliminabili mediante termoterapia.

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UKRAINEAN RESEARCH INSTITUTE OF VITICULTURE AND ENOLOGY, ODESSA

ON THE COMPOSITION, DISTRIBUTION AND HARMFULNESS OF VIRUS DISEASES OF GRAPEVINE IN UKRAINA

P.M. SHTERENBERG, B.N. MILKUS, E.A. BEREZOVSKAYA

The fanleaf group viruses can often be met in Ukrainean vineyards. A strain of this group, the infectional chlorosis virus, is widely spread. It is characterized by the lemonyellow colour of leaves, sometimes with ochre tint. The change of leaf colour is often accompanied by small leaf, short nodes, double nodes, leaf deformation. It seems to be associated with the infection by both, the infectional chlorosis strain and by a strain of fanleaf virus. From other symptoms of virus ethiology one can observe the supression of vines that differ by the shortening of internodes, thinning of shoots, small leaf, hardness of leaf blade with chlorotic spots between veins, the appearance of necrosis and abundant side shoot growth. Often such symptoms appear on the stock growth of vines, especially on the Riparia × Rupestris 3309 variety.

In some years the symptoms of leaf roll appear with different degree of severity. These symptoms consist of the early appearance of autumn colour of leaves. The diseased vines differ by imperfect maturity and low sugar content in them.

To confirm the virus nature of the disease we applied the following methods: green grafting of eyes from the tested vine on a stock-indicator, and the implantation of an eye from a variety-

indicator on the tested vine; the analysis of the presence of endocellular cordons in mature shoots and conducting roots; the mechanical inoculation of virus on herbaceus test plants. In a number of cases for the identification of a virus we also applied electron microscopy and serologic analysis using the antisera, supplied from France and German Federal Republic.

The symptoms of the infectional chlorosis, transfered from diseased scion to a healthy stock, appeared 25-30 days after grafting, on late grafts on the next year, and in some cases only on the third year. The symptoms of infectional chlorosis and fanleaf have transfered on all the stock varieties tried. The vines could be distinguished by the deformation of leaf blade, small leaf, short nodes and double nodes. On old leaves the symptoms can mask afterwards, while on young ones the yellowing may be strong, even if they due to abundant rains appear in the end of summer. The disease strengthens with time. No cases of recovery were observed. The isolates from the infected vines differ considerably by the degree of their depression.

All the tried varieties proved to be indicators of viruses of infectional chlorosis and fanleaf group, namely: Riparia × Rupestris 101-14, Riparia × Rupestris 3309, Kober 5BB, Rupestris du Lot, Richter 57, Rkatsiteli × Riparia 14, 101-14 × Amur, Mtsvane × Riparia 11, Hanses, Traminer and others. Reisolated from these varieties after grafting on other ones had shown analogous symptoms.

The grafts in which the buds from the vines infected by lime chlorosis had been used as scions, gave healthy shoots with green leaves and vigorous growth. These plants remained healthy in the following years also. The symptoms of mutational albinism in some seedlings, taken from the plant breeding department of the Institute, preserved in their vegetative progeny, but were not transfered to their stocks.

The forming of endocellular cordons is the characteristic feature of the viruses of infectional degeneration group. Though some authores argue against this statement, A. Vuittenez, speaking at the 4-th International Conference of vine viroses (1970) claimed, that the study of endocellular cordons remains perfectly acceptable method of diagnosis even nowdays. The isolates, prepared in our laboratory, induced the forming of endocellular cordons. From all the stock-plants tried the most intensive forming cordons was

observed in Kober 5BB, 101-14 and Rupestris du Lot, and less intensive in others.

The mechanical transfer of viruses from infected plants proved to be most successful on: *Chenopodium amaranticolor, Ch. quinoa, Ch. album,* the reaction being the best on *Ch. album* and *Ch. quinoa.*

The shoots from vines characterized by the supression of growth, but without yellow colour of leaves, being grafted on some stock varieties, mainly Rupestris du Lot, transfered to them the symptoms of veinclearing of 3-4-th order veins. The most intensive clearing appeard at the beginning of summer. All the pointed above symptoms are nowdays attributed to a virus, different from fanleaf, to that of « marbrure » (MB). The difference consist in the fact, that the MB virus does not react with the fanleaf antiserum, does not induce the forming of endocellular cordons, does not infect test-plants etc. (Vuittenez, 1970). Being latent in many vine and stock varieties, MB virus transferes to *Vitis Rupestris* and its hybrids while grafting.

To check the possibility of the latent presence of virus in the mother plantation of grapevine scions of 15 European varieties, 3-5 vines had been selected among the most vigorous and the same number among those of medium vigour. In may-june 1971 from each selected vine several buds had been grafted on du Lot variety. The symptoms on stocks and scions were analysed on the 15.IX.1971 and 19.IV.1972. The shoots of the tested scion developed normally, but on stocks in the year of grafting the symptoms were observed on 9 of 15 varieties, thus the virus had been transfered on 47% of vines and 40% of grafts. In 1972 this number has grown to 47% vines and 55% grafts (Table 1).

From the data, given in the table no correlation between the vigour of vines and transfer of symptoms from scion to stock-indicator can be seen.

Other data also confirm broad distribution of MB virus in our vineyards.

The testing of symptomless vines, given by clone ancestors and of breeding forms, by grafting on du Lot had also discovered 5% of infected vines in the year of grafting. The next year this number went up to 70% of MB. Analogous results had been received while testing on du Lot symptomless vines from multiplication parcels and also the vines with the symptoms of chlorosis, of degradation due to soil deficiency in many farms.

TABLE 1.

The transfer of marbrure virus by grafting of buds from symptomless vines to stock vines (grafted in 1971)

The condition of donor-vines	The number of tested				% symptoms transfer					
					1971			1972		
	varieties	vines	grafts	survived	varieties	vines	grafts	varieties	vines	grafts
	RUPESTRIS DU LOT									
Vigorous	15	36	138	79	47	36	42	66	52	63
Medium	15	35	103	57	60	37	37	60	43	37
Total	15	71	241	136	60	36.6	40.2	80	47	55

The high percent of MB infection in our vineyards is not surprising. Analogous cases had been reported by BOVEY (1970) and BOUBALS (1971).

It was interesting to clear out the reciprocal influence of MB and the infectional degeneration group viruses. For this purpose the buds from vines infected with MB were grafted on vines, infected with infectional chlorosis virus and vice versa. The study shows the possibility of joint infection of grapevine by both viruses, the MB virus being somehow strengthened by that of infectional chlorosis. On du Lot infected by MB and then by panachure, the veins of 3-4th order and neighbouring tissues appeared to be coloured lemon-yellow. After grafting on du Lot of the shoots from suspicious vines often the same symptoms were observed. In such cases cordons also could be found in du Lot shoots. Thus such symptoms correspond to the mixed infection by MB and infectional chlorosis viruses.

The distribution of viruses and their harmfulness becomes more and more significant, especially due to the growing exchange of planting material.

Passing over to grafted culture, connected with *Phylloxera* infection, also favoures the spreading of viruses, because in grafted plant the infection can pass to healthy biont from interection of the viruses of infected stock and scion. The growing sensitivity of one component of the graft due to its joining with the infected less sensitive biont can also be significant for the strenthening of the depression of the plant.

Acknowledgments

We express our gratitude to Prof. A. Vuittenez and Prof. R. Bercks, who kindly supplied us with the antisera.

RIASSUNTO

Composizione, distribuzione e nocività delle virosi della vite in Ucraina

Virosi del gruppo del « fanleaf » si possono trovare frequentemente nei vigneti dell'Ucraina. In particolare un ceppo di questo gruppo, quello denominato « infectional chlorosis », è molto diffuso. Sono state effettuate con successo trasmissioni sperimentali a varie specie da portinnesto e a piante erbacee (*Chenopodium album*, *C. amaranticolor* e *C. quinoa*). La presenza di cordoni endocellulari è stata notata in modo particolare in piante malate di Kober 5BB, 101-14 e Rup. du Lot.

Mediante trasmissioni per innesto su Rup. du Lot è stata messa in evidenza una elevata frequenza (fino al 70%) del virus della « Marbrure ». Sono pure stati studiati gli esiti di infezioni combinate di « Marbrure » e « infectional chlorosis ».

I.N.I.A. - AVDA. PUERTA DE HIERRO s/n, MADRID-3 - SPAIN

FURTHER RESEARCH ON THE DETECTION OF GRAPEVINE VIRUSES IN SPAIN

A. PEÑA-IGLESIAS and PILAR AYUSO

Grapevine fanleaf virus (Hewitt *et al.*, 1970; Vuittenez, 1970) was detected in two regions of Spain (Almería and Jerez de la Frontera) and the ultrastructure of *Chenopodium quinoa* plants infected with this virus was studied (Peña-Iglesias and Rubio Huertos, 1971). The presence of virus-like particles forming rows and surrounded by a tubular membrane with parallel arrangements were located in the cytoplasm of the infected cells. Some of these tubules containing the virions were located within plasmodesmata. Tubular inclusions with hollow particles were found in the nucleoplasm of nuclei of some cells.

The ultrastructure described has some similar point with the one studied by Gerola, Bassi and Belli (1969). They observed similar virus-like particles aligned in single rows but no tubular membrane surrounding the virions was seen nor any other similar bundle tubular inclusion arrangement which we have seen with GFV isolates.

Just in 1971 (Peña-Iglesias and Ayuso, 1971), but subsequently to Peña-Iglesias and Rubio-Huertos works, we identified

Grapevine fanleaf virus (GFV) and its strain Grapevine yellow mosaic (GYMV) infecting vines in the same two regions of Spain on the basis of mechanical transmissions, physical properties, histology, serology and electron microscopy. At that time we began the graft transmission studies and the identification of GYMV as cause of a disease looking like yellow mosaic (parrales canarios).

At Jerez de la Frontera and in the same vineyards where we collected the samples, the vector *Xiphinema index* was found (Tobar and Peman, 1970; Alfaro García, 1971).

Now the results of the graft transmissions on woody indicators (*Rupestris* du Lot, LN-33 etc.) have shown that GFV and GYMV are the causes of these diseases.

We have also detected GFV and GYMV in other regions in Spain. For instance: GFV has been found in Rioja, Valladolid, Requena, Cataluña, La Mancha, Montilla. GYMV were detected in Albacete, Montilla and La Mancha.

Grapevine fleck disease has been found in combination- with other viruses in some spanish varieties or infecting other ones in a latent form.

Grapevine leaf roll disease has been also detected. We have not done yet sufficient work to know its incidence in Spain.

RIASSUNTO

Ulteriori ricerche sui virus della vite in Spagna

Si è accertata in Spagna la presenza delle seguenti virosi della vite: arricciamento (fanleaf), mosaico giallo (yellow mosaic), accartocciamento (leafroll) e « fleck ».

Studi ultrastrutturali, effettuati al microscopio elettronico su sezioni di foglie di *Chenopodium quinoa* infettato con il virus dell'arricciamento (GFV), hanno rivelato la presenza nelle cellule infette di particelle virali allineate e racchiuse in tubuli. Tubuli contenenti il virus sono stati osservati pure nei plasmodesmi. Tubuli vuoti sono risultati presenti nel nucleo.

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INSTITUT DE VITICULTURE, PLEVEN (BULGARIE)

SUR LA SYMPTOMATOLOGIE DE LEGNO RICCIO (BOIS STRIÉ) DE LA VIGNE EN BULGARIE

PENKA ABRACHEVA

La maladie legno riccio (bois strié), décrite par Ciccarone (1962), Graniti et Ciccarone (1961), Graniti (1964), Graniti et Martelli (1965), Martelli *et al.* (1967), Lehoczki *et al.* (1968) et Hewitt (1968) a été constatée en Bulgarie en 1969 (Abracheva, Tzvetanov, 1970) dans un vignoble à cinq ans d'âge, près de Pleven, chez le cépage Bolgar, planté sur le porte-greffe Rupestris du Lot.

MATERIEL DE L'EXPÉRIMENTATION

Les études sur la symptomatologie de la maladie legno riccio sont réalisées pendant la période 1969-1972 sur les souches infectées par voie naturelle. Nous avons examiné 24 cépages industriels, entre lesquels les cépages Bolgar, Dimiat, Rkatziteli, Saperavi, Ugni blanc, Cabernet Sauvignon et les trois porte-greffes, principaux pour la Bulgarie, a savoir Rupestris du Lot, Kober 5 BB et Chasselas × Berlandieri 41 B. Le nombre total des souches contrôlées dépasse 8000. Les contrôles sont réalisés dans les plantations appar-

tenant aux fermes coopératives dans les départements de Pleven, Tarnovo, Roussé et Bourgas, dans les vignobles privés, dans les environs des villes de Vratza, Pleven, Lovetch et Loukovit.

L'âge des souches contrôlées est entre 3 et 15 ans.

RÉSULTATS

En examinant les cépages nous avons constaté que les souches, montrant des symptômes moins visibles sur le bois sont relativement bien développées avec une fructification normale.

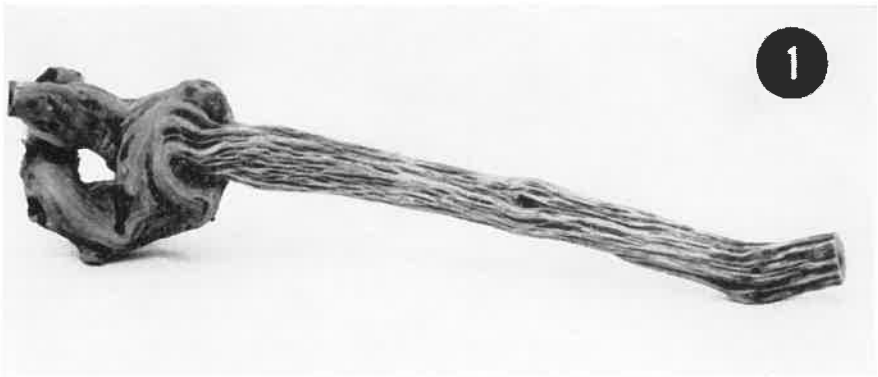
Les souches montrant des symptômes bien visibles sur le bois ont des feuilles petites, d'une forme qui n'est pas caractéristique pour le cépage, asymétriques.

Les sarments sont d'une croissance faible, dont le rendement est bas et de basse qualité.

En enlevant l'écorce nous observons sur la partie ligneuse du porte-greffe des cavités, caractéristiques pour la maladie, situées longitudinalement (fig. 1). Elles sont d'une forme de lancette, d'un centre bien visible. Au centre les cavités sont plus profondes et vers les bords moins profondes. La profondeur au centre mesure parfois jusqu'à 5 mm, mais ordinairement elle atteint entre 1.5 et 3 mm. La longuer des cavités est d'un mm jusqu'aux 2-3 mm, mais on rencontre même plus longues — 4-5 mm.

En cas du fusionnement des deux ou plusieurs de ces cavités on voit des bandes d'une profondeur de 6-7 cm.

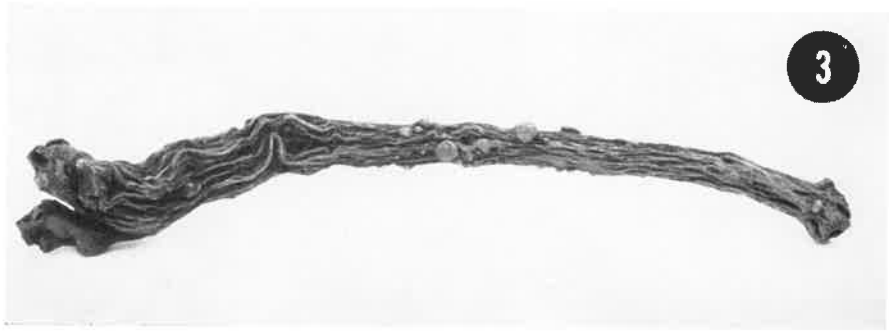
En plus, outre les cavités montrant une forme de lancette nous avons constaté même des cavités aux bords et fond arrondis. Dans le cas de ce type de cavités on n'apperçoit pas des différences dans leur profondeur au centre et aux bords.



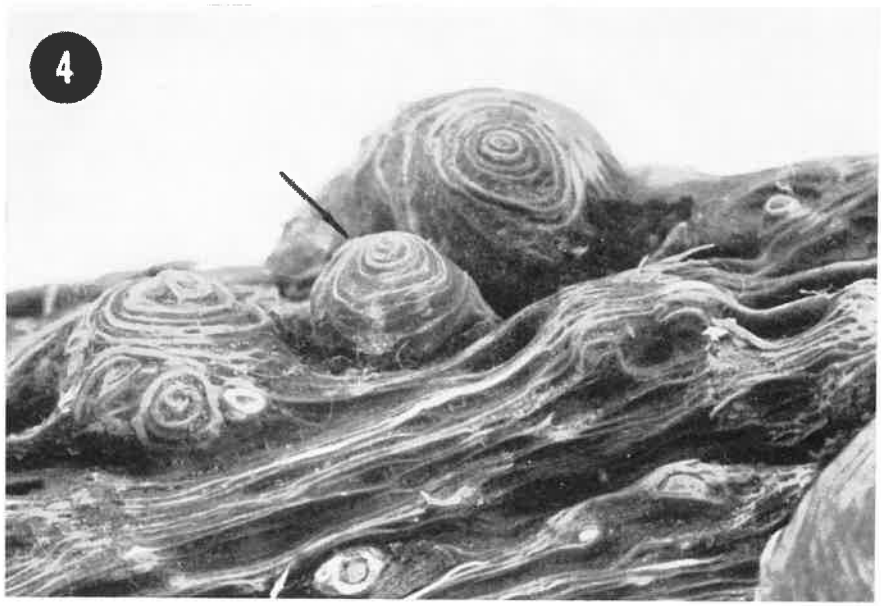
Pendant 1971 nous avons constaté des symptômes nettement visibles de legno riccio sur la tige aérienne des souches à haute conduite, à 7 ans d'âge, appartenant aux cépages Bolgar et Ubilej (fig. 2). Les cavités avaient une forme de lancette, elles entouraient la tige, formant un anneau à l'hauteur au plus de 40 cm au dessus de la soudure.

Nous n'avons pas encore des données sur la sensibilité des cépages au legno riccio, mais en se basant sur les premiers résultats de nos études pour la détermination du degré de la propagation des maladies à virus de la vigne dans certaines régions de la Bulgarie du Nord, le porte-greffe Rupestris du Lot montre un haut pourcentage de contamination. Les plantations crées sur cet porte-greffe sont aussi en haut degré contaminées. Chasselas × Berlandieri 41 B et Kober 5 BB pratiquement sont indemnes de legno riccio et les plantations faites sur ces deux porte-greffes sont moins contaminées.

En été de 1972 chez un petit nombre de souches, montrant des symptômes bien visibles de legno riccio nous avons constaté des formations tumeureuses (fig. 3) qui proviennent du bois. Elles ont une surface polie, aux cercles concentriques bien découpés qui sont



étalés autour d'un centre dans le cas des formations plus petites et autour de 2-3 points centraux dans le cas des formations plus grandes. Les formations bien développées sont d'une forme arrondie, ressemblante à l'haricot, d'une diamètre du 5 aux 20 mm. Elles sont liées à la tige de la souche par un rétrécissement court. Les formations les plus petites qui sont en cours de formation ont une forme d'une coupole, d'une base large, dont la partie supérieure est rétrécie (fig. 4).



Les formations tumeureuses sont entièrement couvertes par l'écorce et on peut bien les voir après leur écorcage.

Les coupes faites ont montré que les formations citées ne sont pas causées par des insectes.

Au cours de l'examen de plus de 8000 souches dans les différents départements et plantations nous n'avons jamais trouvé des formations tumeureuses chez les souches saines. Cet état de choses nous fait penser que leur apparition est liée probablement à la maladie legno riccio.

CONCLUSION

Les observations sur la symptomatologie de legno riccio des feuilles en Bulgarie ont montré que les cavités, disposées longitudinalement sur la partie ligneuse radicale et la partie aérienne, typiques pour cette altération peuvent avoir une forme de lancette ou bien d'un petit lit aux bords et au fond arrondis.

Dans quelques cas chez les souches contaminées de legno riccio on peut constater des formations tumeureuses provenant du bois. Chez un grand nombre de souches examinées on n'a jamais constaté de pareilles formations chez les plantes saines et la liaison de legno riccio avec l'apparition des formations tumeureuses n'est pas prouvée.

Les contrôles faits dans certaines régions viticoles en Bulgarie du Nord montrent qu'en comparant les trois porte-greffes utilisés, le plus largement, a savoir Rupestris du Lot, Kober 5 BB et Chasselas × Berlandieri 41 B, c'est le porte-greffe Rupestris du Lot montrant le plus haut pourcentage de contamination, tandis que les deux autres porte-greffes sont moins contaminées.

RIASSUNTO

Sulla sintomatologia del « legno riccio » della vite in Bulgaria

Il « legno riccio » è stato osservato per la prima volta in Bulgaria nel 1969. Gli studi sulla sintomatologia della malattia sono stati effettuati durante il periodo 1969-1972 su viti appartenenti a 24 varietà e ai tre principali

portinnesti per la Bulgaria: Rupestris du Lot, Kober 5BB e 41 B. Sollevando la corteccia delle viti malate, si potevano osservare sulla parte legnosa del portinnesto numerosi solchi longitudinali, la cui lunghezza variava da 1 a 5 mm e la cui profondità arrivava fino a 5 mm. Le viti colpite presentavano anche foglie piccole e asimmetriche, tralci deboli, produzione scarsa e di qualità scadente. Nel 1971 sono stati osservati sintomi evidenti di « legno riccio » anche sulla parte aerea del tronco di viti appartenenti alle varietà Bolgar e Ubilej. Per quanto riguarda i portinnesti, Rupestris du Lot ha manifestato la più alta percentuale di infezione, mentre Kober 5 BB e 41 B risultano praticamente indenni.

L'A. riferisce anche di aver riscontrato su alcune viti affette da « legno riccio » delle formazioni tumorali, provenienti dal legno, di forma arrotondata. Le più grosse avevano un diametro variabile dai 5 ai 20 mm e presentavano dei rilievi circolari concentrici intorno a 2 o 3 punti centrali. Queste formazioni tumorali diventavano visibil solo dopo aver tolto la corteccia.

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FEDERAL AGRICULTURAL RESEARCH STATION OF LAUSANNE, CH 1260 NYON (SWITZERLAND)

STEM PITTING OF GRAPEVINE IN SWITZERLAND

R. BOVEY and J.-J. BRUGGER

A grapevine disease very similar to « legno riccio » (GRANITI, 1964; GRANITI and MARTELLI, 1966; MARTELLI *et al.*, 1967; LEHOCZKY, 1972) has been recorded for the first time in Switzerland by NICOLLIER (1970). It occurs on two local varieties. Païen (in French) or Heida (in German) is grown mainly in the region of Viège in Valais and is similar to the Savagnin blanc grown in the French Jura. Humagne (or Umagne) is also grown in Valais. Both varieties appear to be almost entirely contaminated. As long as they grew on their own roots, these varieties were normal. The disorder has become apparent because of the necessity of cultivating on *Phylloxera* resistant american rootstocks. The first plantings of Païen grafted on Kober 5 BB were made around 1960. Stem pitting symptoms were noticed from 1966, only on grafted vines. The vineyards planted in the same region with Pinot noir, Gamay, Silvaner or Riesling × Silvaner appear normal.

SYMPTOMS

The symptoms are very similar to those described in Italy and Hungary for «legno riccio». The typical pitting of the surface of the wood (fig. 1), reproduced in an inverted pattern on the inner face of the bark when this is peeled off, appears only on the rootstock. The graft union is swollen. The bark is rougher and

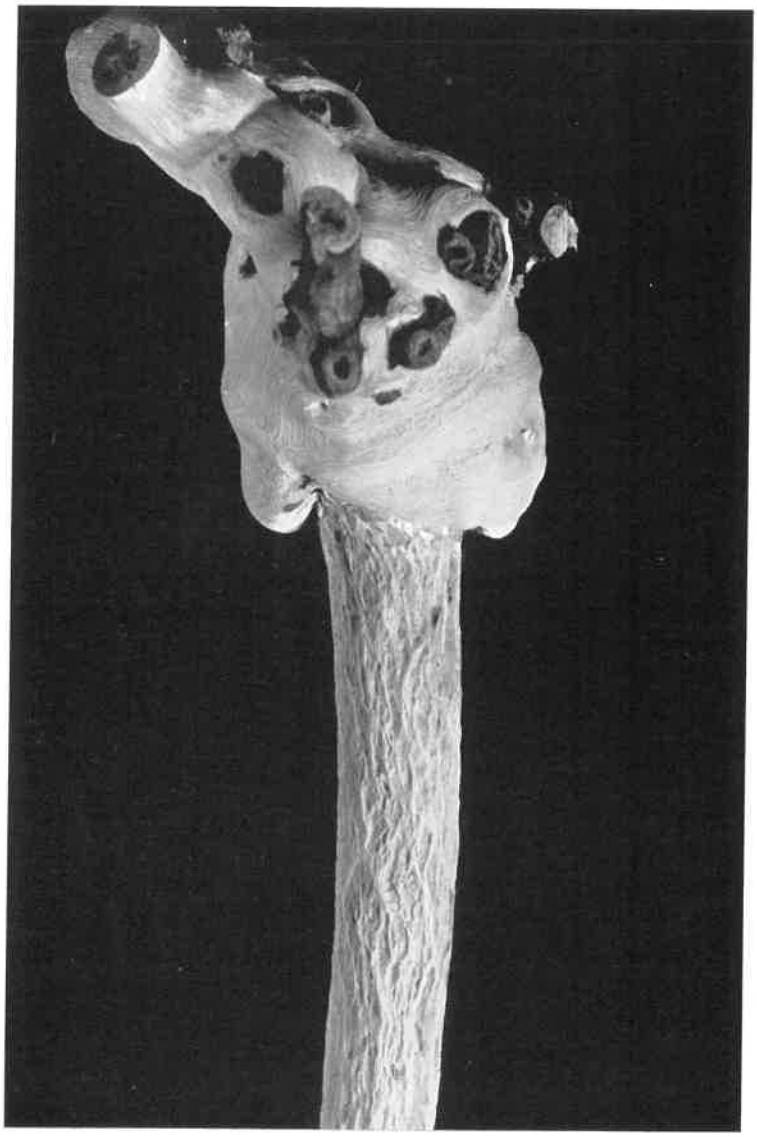

FIG. 1 - Symptoms of stem pitting on a vine-stock of Païen. The bark has been peeled off to show the pitted surface of the wood of the root-stock Kober 5 BB. Note the swelling at the graft union, and the absence of pitting on the scion variety Païen.

thicker than normal, and has a softer consistency. There is little o. no delay in bud bursting in spring. Affected vines often bear slightly rolled leaves, but they show no symptom of fanleaf. They lack in vigour and die back in a few years. In the nursery, the graft take is normal, but there is commonly a loss of 50% during the first year.

ATTEMPTS TO IDENTIFY THE AGENT OF THE DISEASE

Graft transmission: The disease has been easily reproduced in glasshouse conditions by grafting scions from diseased plants onto the rootstocks Kober 5 BB, 3309, 99 R and *Vitis rupestris* du Lot. Indexing by chip budding was made in 1971 on *Vitis rupestris* St. George, Chardonnay, Kober 5 BB, Gamay Rouge de la Loire and LN 33. Up to now, with most of the sources of Païen and Humagne tested, only leafroll symptoms on Gamay Rouge de la Loire have been observed. This needs confirmation in the second year (1973).

Mechanical inoculation: Several attempts to transmit a virus from leaves of vines affected by stem pitting to *Chenopodium quinoa* were unsuccessful. In the same conditions (grinding the leaves in 2,5% nicotine), fanleaf virus was transmitted easily from leaves of vines growing in the same region and showing symptoms of fanleaf or yellow mosaic.

Serology: 10 g of leaves taken the same day (17.5.73) from vines showing (a) symptoms of stem pitting (Païen), (b) symptoms of fanleaf and/or yellow mosaic (Gamay), (c) no symptoms (Savagnin blanc), and growing in two vineyards in central Valais were ground in 50 ml of 2,5% nicotine. The liquid was clarified by centrifugation at 5000 r.p.m. during 15 min, and the supernatant centrifugated at 34000 r.p.m. for 2 hours. The pellet was resuspended in 5 ml of nicotine and used for serological tests with anti-fanleaf antiserum, using Ouchterlony's agar double diffusion method. No reaction occurred with (a) and (c) extracts, whereas a positive reaction was observed with extract (b).

Electron microscopy: All attempts to detect virus particles from leaves or bark of diseased plants, with the electron microscope by the negative staining technique or by sectioning the bark in pitted regions, have so far been negative.

DISCUSSION

Stem pitting or « legno riccio » of grapevine has been recorded in the following countries: Italy (Graniti, 1964; Graniti and Martelli, 1966, 1970), Hungary (Martelli *et al.*, 1967; Lehoczky *et al.*, 1968; Lehoczky, 1972), Israël (Hewitt, 1968), Switzerland (Nicollier, 1970), Bulgaria (Abrasheva and Tsvetanov, 1970), California (Hewitt and Neja, 1971), Greece (Agrios, 1971), South Africa (Engelbrecht and Nel, 1971), France (Legin, 1972).

As the disease was often found associated with fanleaf virus, it has been suggested (Hewitt, 1968; Graniti and Martelli, 1970; Hewitt and Neja, 1971) that this virus could play a part in the etiology of stem pitting. Graniti and Martelli (1966, 1970) put forward the hypothesis that stem pitting might be due to the combined effect of two viruses, symptomless when infecting the rootstock and scion varieties separately, but virulent when they occur together after grafting. Lehoczky *et al.* (1968) showed however that stem pitting symptoms develop on non grafted grapevines. Legin (1972) has found a correlation between stem pitting and leafroll, but not between stem pitting and fanleaf or marbrure (fleck). Engelbrecht and Nel (1971) also conclude from their indexing experiments that stem pitting and fanleaf are not related.

From our preliminary experiments, it can be concluded that fanleaf virus is very unlikely to play any role in the etiology of the stem pitting disease occuring in Switzerland on the two varieties mentioned in this paper. The agent of grapevine stem pitting has been probably latent for a long time in these varieties before they were grafted on american rootstocks. Nicollier (1970) mentions that difficulties in grafting the variety Païen were already noted in 1927. The disease seems to be due to the infection of a sensitive rootstock by a pathogen that is latent in the scion variety, a situation very similar to that of apple stem pitting and stem grooving viruses.

As the viral nature of this disease has not yet been demonstrated, it would probably be better to consider it as a viruslike disease. Also, we cannot rul out the possibility that more than one pathogen can cause stem pitting on grapevine.

Acknowledgments:

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RIASSUNTO

Il « legno riccio » della vite in Svizzera

Una malattia della vite molto simile al « legno riccio » è stata riscontrata in Svizzera su due varietà: Païen e Humagne. Allo scopo di identificare l'agente della malattia sono state effettuate prove di trasmissione su viti indicatrici e su *Chenopodium quinoa*, esami sierologici e ricerche al microscopio elettronico. Tutte queste prove però non hanno permesso di identificare l'agente responsabile. Sembra che il virus dell'arricciamento non giochi nessun ruolo nell'eziologia del legno riccio. La malattia sembra si manifesti solo quando marze di una delle due varietà sopracitate, dove il patogeno probabilmente si trova allo stato latente, vengono innestate su un portinnesto sensibile.

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ISTITUTO DI PATOLOGIA VEGETALE DELL'UNIVERSITÀ DI SASSARI, ITALIA

RICERCHE INTORNO A COMPORTAMENTI PARTICOLARI DELLE VITI CON SINTOMI ASSIMILABILI AL « LEGNO RICCIO » IN SARDEGNA (°)

R. GARAU, U. PROTA e O. SERVAZZI

Un'alterazione della Vite, identificabile col « legno riccio » (Graniti e Ciccarone, 1961), di cui esisteva una precedente segnalazione (Graniti e Martelli, 1970), è stata recentemente descritta in Sardegna (Garau *et al.*, 1973).

Le notizie riguardanti la sintomatologia di questa malattia (cfr. Graniti e Martelli, l.c.), indicano una costante associazione delle alterazioni interne a carico del legno, con una vegetazione stentata e un aspetto rachitico delle piante che possono presentare, oltre a sintomi riferibili alle virosi del gruppo *fanleaf*, delle nodosità in corrispondenza del punto d'innesto.

Poiché, per altro, avevamo notato che esistevano casi in cui tale associazione non era pienamente confermata, abbiamo ritenuto

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utile approfondire le indagini nel tentativo di stabilire un più preciso quadro sintomatologico della malattia.

Un altro tema della ricerca è stato quello d'individuare una eventuale scala di sensibilità dei portinnesti e delle cv fruttifere, in base alle reazioni interne mostrate da ciascuno di essi.

Infine, estendendo le osservazioni ad alcuni casi specifici, si è tentato di ricavare indicazioni sul decorso della malattia e particolarmente sul suo presunto esito letale.

I. RELAZIONE TRA SINTOMI INTERNI A CARICO DEL LEGNO ED ALTRI SINTOMI (FANLEAF, SVILUPPO RIDOTTO E NODOSITÀ)

L'analisi venne condotta con le modalità descritte nella precedente nota (Garau *et al.*, l.c.), su 525 viti appartenenti a diverse specie ed ibridi portinnesto, innestate con varie cv fruttifere e distribuite in diversi vigneti dell'Isola.

I risultati, sintetizzati nelle tabb. 1 e 2, mostrano, nel complesso, che i sintomi di *fanleaf*, l'aspetto rachitico o lo sviluppo comunque ridotto e le nodosità a livello del punto d'innesto, presi singolarmente o tra loro variamente combinati, non possono essere considerati specifici per una generale caratterizzazione delle piante colpite. Infatti le percentuali relative alle piante che presentavano sintomi esterni combinati con quelli interni, non differiscono sostanzialmente dalle percentuali relative alle piante con solo sintomi esterni. Ciò mette in evidenza il fatto che, nello stesso ambiente, aspetti e comportamenti perfettamente identici ai sopra descritti, possono risultare indipendenti da quelli eventualmente legati alla presenza dello *stem-pitting*.

Delle tre situazioni esaminate, la presenza nelle piante di sintomi di *fanleaf* e di nodosità a livello dell'innesto è da ritenere la meno specifica per quanto riguarda l'individuazione, su base sintomatologica esterna, delle piante colpite da *stem-pitting*. La riduzione dello sviluppo, da sola o con sintomi di *fanleaf*, sembrano più frequentemente associate con le piante colpite dalla malattia, tanto più quanto più grave è la sintomatologia interna a carico delle piante. Questo accostamento è risultato particolarmente frequente per gli ibridi *V. Berl.* × *V. rup.* 140 e *V. Berl.* × *V. rip.* K.5 BB e 34 E. Un carattere, invece, molto comune alle piante colpite da *stem-pitting*, già ricordato da vari

TABELLA 1.

Percentuali di viti rispettivamente con sintomi di *fanleaf*, con sviluppo ridotto e con nodosità, riferite ai totali di piante presentanti o meno *stem-pitting*.

	Senza *stem-pitting*	Con *stem-pitting*			
		intensità			Totale
		1	2	4	
Fanleaf	56.6	54.6	65.1	47.0	56.3
Sviluppo ridotto	43.5	48.7	51.8	60.8	51.7
Nodosità	43.0	40.3	57.8	49.8	46.9

TABELLA 2.

Distribuzione delle piante appartenenti alle diverse specie ed ibridi portinnesto ed alle cv fruttifere, in base ai rispettivi indici d'intensità, e relativo indice medio.

Soggetti portinnesto	N° viti intensità			tot.	Indice medio	Cv fruttifere	N° viti intensità			tot.	Indice medio
	1	2	4				1	2	4		
Berl. × rup. 140	14	15	7	36	2.00	Nuragus	15	3	2	20	1.45
» » 1103	35	27	16	78	1.96	Sangiovese	4	1	—	5	1.20
Berl. × rip. K.5BB	5	4	2	11	1.91	Cannonau	1	—	—	1	1.00
Rupestris du Lot	26	17	10	53	1.87	Monica	1	—	—	1	1.00
Berl. × rip. 420A	15	2	5	22	1.77	Pietro	1	—	—	1	1.00
» » 34E	17	9	4	30	1.70	Taloppo	1	—	—	1	1.00
Berl. × rup. 17.37	23	3	6	32	1.66	Vermentino	2	—	—	2	1.00
» » 779	14	5	1	20	1.40						
Berl. × rip. 225	5	1	—	6	1.17						

AA. (Graniti e Martelli, l.c.; Lehoczky, 1972), è l'evidente ritardo nella ripresa vegetativa primaverile. Tuttavia è necessario precisare che le notevoli differenze iniziali dello sviluppo scompaiono con l'avanzare della stagione, sicché, in estate, unicamente in base a questo carattere, riesce difficile il riconoscimento delle piante colpite.

Infine, è importante segnalare che si è potuto accertare la presenza di sintomi anche gravi di *stem-pitting*, in percentuali non molto elevate, ma non trascurabili, di piante il cui aspetto generale era apparentemente normale.

II. INDAGINI SULLA SENSIBILITÀ ALLO STEM-PITTING DI VARI SOGGETTI PORTINNESTO E CV FRUTTIFERE

Le indicazioni sulla sensibilità dei vari soggetti vennero ottenute riferendo ad un punteggio convenzionale le stime dell'intensità dei sintomi interni osservati su ciascuna pianta e ricavandone poi un indice medio (*): vedi tab. 2.

E' risultato che i diversi portinnesti saggiati hanno manifestato, indipendentemente dalla cv fruttifera, un'intensità di sintomi molto variabile, ma con valori medi che consentono una certa differenziazione. Sembrerebbe infatti che i soggetti *V. Berl.* × *V. rup.* 140 e 1103, *V. Berl.* × *V. rip.* K.5 BB e *V. rupestris* du Lot siano portati a manifestare forme più gravi di *stem-pitting*, rispetto ai soggetti *V. Berl.* × *V. rip.* 420 A e 34 E e *V. Berl.* × *V. rup.* 17.37 che mostrerebbero forme intermedie e ai soggetti *V. Berl.* × *V. rup.* 779 e *V. Berl.* × *V. rip.* 225, sui quali, invece, i sintomi apparirebbero con una minore intensità. Su questo argomento non sono disponibili, nella letteratura, notizie dettagliate; qualche indicazione è comunque fornita da Graniti e Martelli (1970) e riguarda la maggiore sensibilità riscontrata nei soggetti *V. Berl.* × *V. rip.*

(*) Ottenuto mediante la formula $I = \frac{1n_1 + 2n_2 + 4n_4}{N}$, dove 1, 2 e 4 sono gli indici d'intensità e rappresentano rispettivamente la presenza di una rugosità lieve, di una rugosità ben evidente con cordoni legnosi rilevati e di una rugosità molto forte, con grossi cordoni e talvolta appiattimento del fusto; n_1, n_2 e n_4 il numero delle piante presentanti tali sintomi e N il numero totale delle viti saggiate.

420 A e *V. rupestris* du Lot quando innestati rispettivamente con le cv fruttifere Primitivo di Gioia e Moscatello. Tra le cv fruttifere solo la Nuragus ha presentato sintomi di un certo rilievo.

III. INDICAZIONI SUL DECORSO DELLA MALATTIA E SUL SUO EFFETTO LETALE

E' ammesso che le viti affette da *stem-pitting*, oltre a presentare una vegetazione stentata e una produzione ridotta, vanno incontro ad un progressivo declino che si conclude con la morte delle piante in un tempo più o meno breve (Graniti e Martelli, 1970; Lehoczky, 1970).

Le indagini da noi condotte per più anni consecutivi, su un notevole numero di viti colpite da *stem-pitting* e che avevano sempre presentato una vegetazione stentata, ci hanno consentito di rilevare che, nel nostro ambiente, la morte prematura dei ceppi malati non è una condizione generale e nemmeno tanto frequente. Nella maggioranza dei casi le piante riprendevano la vegetazione, seppur con un notevole ritardo nel germogliamento e ricostituivano una chioma che, grosso modo, non si differenziava molto da quella dell'anno precedente.

Abbastanza frequenti sono risultati, invece, i casi di morte improvvisa di certe piante che avevano sempre presentato una vegetazione rigogliosa ed una normale produzione.

Con un'indagine condotta durante l'estate del 1972, in diversi vigneti della Sardegna centro-settentrionale, si è rilevato che circa il 75% delle viti colpite da tale rapido declino presentava una forma piuttosto grave di *stem-pitting*. Le viti appartenenti alle cv Cannonau, Carignano e Pascale di Cagliari, tutte innestate su *V. rupestris* du Lot, avevano un'età compresa tra i 15 ed i 24 anni e, in precedenza, non avevano mostrato segni di sofferenza.

La spiegazione di questi fenomeni può essere ricercata nel disordine dei tessuti, specialmente di quello vascolare (Graniti, 1964) che impedirebbe alla pianta, in particolari condizioni ambientali, un adeguato rifornimento idrico. Naturalmente in queste circostanze soccomberebbero più facilmente quelle piante che, favorite da precedenti stagioni propizie e nonostante le alterazioni indotte dalla malattia, erano riuscite a formare una folta chioma.

All'insufficiente rifornimento idrico sarebbero da attribuire

anche i casi, descritti da Lehoczky (l.c.), di mancato germogliamento e di moria verificatisi in Ungheria.

A nostro avviso, infine, la sopravvivenza per lungo tempo di molte piante colpite da *stem-pitting*, anche in forma grave, non contrasta con quanto sopra esposto, e può essere spiegata principalmente con la modesta esigenza alimentare correlata al ridotto sviluppo epigeo, per cui tali piante sarebbero in grado di superare senza gravi danni eventuali periodi di crisi.

RIASSUNTO

In Sardegna, le viti colpite da *stem-pitting* possono presentare sintomi di *fanleaf*, sviluppo ridotto e nodosità a livello dell'innesto; ma questi sintomi esterni non consentono una specifica caratterizzazione delle piante malate. Lo sviluppo ridotto da solo o con sintomi di *fanleaf* sembra essere più frequentemente associato coi sintomi di rugosità del legno.

I portinnesti e le cv fruttifere presentano una notevole variabilità nell'intensità dei sintomi interni: i soggetti *V. Berl.* × *V. rup.* 140, 1103, *V. Berl. V. rip* K.5 BB e *V. rupestris* du Lot e la cv Nuragus sembrano manifestare le forme più gravi.

Le piante colpite da *stem-pitting* e che presentano uno sviluppo ridotto non vanno incontro, di solito, ad una morte prematura. Più frequenti sembrano, invece, i casi di morte improvvisa di piante affette dalla rugosità interna, ma presentanti una buona vegetazione.

SUMMARY

In Sardinia, vines affected by stem-pitting can present symptoms of fanleaf, reduced growth and enlargements at the graft level; but these external symptoms do not permit the characterizing of the diseased plants. The reduced development alone or with symptoms of fanleaf appears to be, nevertheless, more frequently associated with the symptoms of rugose-wood.

Rootstocks and fruit-bearing varieties present a considerable variability in the seriousness of internal symptoms: hybrids *V. Berl.* × *V. rup.* 140, 1103, *V. Berl.* × *V. rip.* K.5 BB and *V. rupestris* du Lot, and variety Nuragus, seem to show the seriousest forms.

Stem-pitting affected plants, presenting also a reduced growth, usually do not go to an untumely death. More frequent, on the contrary, seem to be the cases of sudden death of rugose wood affected plants showing a good development.

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GRUPPO DI RICERCA SUI VIRUS E LE VIROSI DELLE PIANTE (C.N.R.)
c/o ISTITUTO DI PATOLOGIA VEGETALE, UNIVERSITÀ, MILANO

PRESENZA DI UNA MALATTIA DEL TIPO « FLAVESCENCE DORÉE » IN VIGNETI DELL'OLTREPÒ PAVESE

G. BELLI, A. FORTUSINI, R. OSLER, A. AMICI

La « flavescence dorée » (FD) è una grave malattia della vite che da tempo è presente in Francia (Caudwell, 1957) ed è stata successivamente segnalata in Germania (Gärtel, 1965) e in Svizzera (Bovey, 1972). Malattie simili alla FD sono state osservate pure in Israele, in Romania e in Cile (Gärtel, 1970). La FD, che prima era considerata una virosi, è stata recentemente attribuita a micoplasmi (Giannotti *et al.*, 1969; Caudwell *et al.*, 1971).

In Italia, la presenza della FD non è ancora stata accertata, benchè sia stata ipotizzata da Zelger (1964) sulla base di osservazioni sintomatologiche compiute in vigneti dell'Alto Adige. Riteniamo pertanto opportuno segnalare una malattia della vite che negli ultimi anni è divenuta abbastanza frequente nell'Oltrepò pavese e che, stando ai risultati di nostre indagini preliminari, sembra presentare molte analogie con la FD.

OSSERVAZIONI SINTOMATOLOGICHE

La malattia è stata da noi osservata per la prima volta nel 1966 in vigneti siti nei Comuni di Torrazza Coste e di Canneto Pavese; successivamente è comparsa in varie altre zone dell'Ol-

trepò, tanto da indurci a farne oggetto di una nostra ricerca a partire dal 1970.

La varietà Barbera risulta essere la più colpita; la malattia è però frequente anche su Croatina e Uva Rara, quindi su tutte e tre le principali varietà di uva nera da vino coltivate nell'Oltrepò. Per ora non abbiamo dati circa la sua possibile presenza sulle varietà di uva bianca, non avendo ancora effettuato ricerche in proposito.

I sintomi principali della malattia da noi studiata possono essere così riassunti:

— mancanza o difficoltà di germogliamento da parte dell'intera pianta oppure da parte di alcune gemme dei tralci malati; produzione di ricacci basali; necrosi dei tratti apicali dei tralci;

— colorazione rosso-viva delle foglie, evidente soprattutto a fine estate. Detta colorazione interessa spesso l'intera lamina delle foglie di uno o più tralci; talvolta però è limitata a poche foglie o a settori della lamina delimitati dalle nervature che pure diventano rosse e in seguito necrotizzano;

— colatura dei fiori;

— raggrinzimento e quindi disseccamento parziale o totale dei grappoli;

— mancata lignificazione dei tralci, i quali in autunno avanzato restano ancora verdi, totalmente o a tratti. I tralci non lignificati presentano spesso numerose pustole nere che risaltano sul fondo verde; hanno consistenza gommosa e tendono a piegarsi verso il basso dando alla vite un aspetto cadente.

— in molti casi i suddetti sintomi sono presenti soltanto su alcuni tralci della pianta, mentre gli altri presentano aspetto normale.

INDEXING SU BACO 22 A E LN-33

Nel marzo 1971 sono state effettuate inoculazioni per « chip-budding » su talee di Baco 22 A e di LN-33 utilizzando porzioni di tralci prelevati da piante di Barbera e Croatina malate.

I primi sintomi sulle viti indicatrici sono stati osservati a fine settembre dello stesso anno; nell'anno successivo sono divenuti più intensi. I risultati nelle prove di indexing sono schematizzati nella tabella n. 1.

TABELLA 1.

Risultati delle prove di indexing

Inoculo	Saggi su Baco 22 A			Saggi su LN-33		
	N° talee		Sintomi ottenuti	N° talee		Sintomi ottenuti
	sagg.	posit.		sagg.	posit.	
Barbera FD-1	6	3	nanismo, ingialli-menti fogliari, tralci non lignifi-cati	6	4	lievi accartoccia-menti e arrossa-menti fogliari
Croatina RR-1	6	1	(idem, come da Barbera)	6	5	(idem, come da Barbera)

RICERCA DI EVENTUALI VETTORI

Come si è detto, negli ultimi anni si è riscontrato un aumento dei casi di malattia in vari vigneti dell'Oltrepò. Questo fatto, unitamente alle analogie sintomatologiche da noi rilevate fra la malattia in studio e la FD, ci ha indotti ad effettuare indagini sulla possibile presenza di vettori alati prendendo in particolare considerazione le cicaline. Le ricerche svolte a partire dal 1972 nei vigneti infetti ci hanno permesso di individuare finora le specie qui sotto elencate.

specie di cicaline	*caratteristiche*
Erythroneura rhamni Ferr. *Empoasca flavescens* F.	ampelofaghe abituali (VIDANO, 1957-58)
Empoasca decipiens Paoli *Ceresa bubalus* F.	ampelofaghe occasionali (VIDANO, 1964)
Euscelis plebejus Fall. *Macrosteles sexnotatus* Fall.	vettori di corpi riferibili a micoplasmi (MARAMOROSCH *et al.*, 1970; VAN SLOGTEREN e MÜLLER, 1969)
Cicadella viridis L. *Delphacodes* sp. *Mocydiopsis parvicauda* Rib. *Psammotettix striatus* L.	riscontrate sulle erbe infestanti i filari dei vigneti infetti

Finora non si è riscontrata la presenza di *Scaphoideus littoralis* Ball., vettore della FD in Francia (Schvester *et al.*, 1961). Tenendo conto comunque dei risultati ottenuti da Caudwell *et al.* (1970), sono state impostate prove di trasmissione da vite a *Vicia faba* impiegando le specie di cicaline sopra elencate.

OSSERVAZIONI AL MICROSCOPIO ELETTRONICO

A partire dal 1971 sono state esaminate al microscopio elettronico sezioni di foglie, piccioli e corteccia di tralci prelevati sia da viti malate di Barbera, Croatina e Uva rara, sia da Baco 22 A inoculato per « chip-budding » con Barbera malata. Come controllo sono stati esaminati tessuti analoghi di viti senza sintomi delle medesime varietà.

I frammenti di tessuto esaminati erano stati fissati in aldeide glutarica al 3% in tampone fosfato 0,1 M (pH 6,9), quindi postfissati in tetrossido di osmio all'1% in tampone fosfato, disidratati in etanolo, colorati con acetato di uranile e inclusi in araldite (Luft, 1961). Le sezioni sono state ulteriormente colorate con citrato di piombo (Venable e Coggeshall, 1965) ed esaminate con un microscopio elettronico Siemens Elmiskop 1A operante a 80 Kv.

I prelievi di materiale sono stati effettuati nell'ottobre 1971 e nei mesi di luglio, settembre e ottobre 1972. In totale sono stati finora esaminati 25 campioni malati e 8 di controllo.

Le osservazioni effettuate al microscopio elettronico hanno permesso di mettere in evidenza in vari casi, nei campioni prelevati da piante malate, le seguenti alterazioni delle cellule floematiche:

— dilatazioni talora molto marcate della cisterna nucleare;
— presenza di corpi contenenti ribosomi nelle dilazioni suddette;
— notevole concentrazioni di ribosomi nel citoplasma.

Dette alterazioni non sono state riscontrate nei controlli sani. Nei cribri del materiale prelevato sia da piante malate che da piante sane non abbiamo finora osservato corpi sicuramente riferibili a micoplasmi.

DISCUSSIONE DEI RISULTATI

La malattia da noi osservata nei vigneti dell'Oltrepò pavese presenta molte analogie con la FD, specialmente se si considerano il quadro sintomatologico rilevato in pieno campo e i risultati del-

l'indexing su Baco 22 A e LN-33. I sintomi da noi osservati sulle viti malate coincidono infatti con quelli descritti in più occasioni per la FD, specialmente per quanto riguarda le varietà ad uva nera (Caudwell, 1965; Bovey, 1972; Boubals e Caudwell, 1971). Anche le risposte sintomatologiche da noi ottenute su Baco 22 A inoculato sperimentalmente coincidono con quelle descritte da Caudwell (1965) per la FD; altrettanto dicasi per quelle ottenute su LN-33 (Bovey, 1972).

Il fatto di non aver finora riscontrato la presenza di *Scaphoideus littoralis* nei vigneti malati non esclude che ciò possa avvenire in futuro, come non esclude l'esistenza di un vettore diverso; in Svizzera, infatti, *S. littoralis* non risulta presente nelle zone dove è stata segnalata la FD (Bovey, 1972).

La ricerca di corpi riferibili a micoplasmi nei cribri di viti malate ha dato finora esito negativo; Caudwell *et al.* (1971) mettono però in evidenza la difficoltà di osservare tali corpi in piante di vite; mentre risulta relativamente facile il loro ritrovamento in piante di fava infettata da vite mediante il vettore. D'altra parte le alterazioni citologiche da noi osservate sono correlate, secondo alcuni autori (Lombardo *et al.*, 1970; Pellegrini e Gerola, 1970; Amici e Favali, 1972), con infezioni riferibili a micoplasmi.

Sulla base di questi risultati preliminari il nostro lavoro futuro sarà volto soprattutto alla ricerca di un possibile vettore, alle prove di trasmissione a piante erbacee e alle indagini di microscopia elettronica sia su vite che su piante erbacee eventualmente infettate. Ciò allo scopo di verificare se la malattia da noi studiata coincide esattamente con la FD oppure se è da considerarsi piuttosto una malattia affine.

SUMMARY

Occurrence of a disease similar to the « flavescence dorée » in vineyards of Oltrepò pavese

A disease of the grapevine, which is very similar to the « flavescence dorée », was observed in some vineyards of Oltrepò pavese (Northern Italy). The symptoms obtained in transmission tests made by grafting to Baco 22 A and to LN-33 seem to be identical with those described respectively by Caudwell (1965) and by Bovey (1972).

Scaphoideus littoralis has not been found yet in the diseased areas. Other species of leafhoppers are present; transmission tests from grapevine to *Vicia faba* are conducted with these species.

Ultrathin sections of leaves, petioles and young shoots of diseased grapevines were examined under the electron microscope: in some of the samples were observed cell alterations which other authors have found associated with mycoplasma-like bodies.

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CENTRE DE RECHERCHES - INRA
STATION DE PATHOLOGIE VÉGÉTALE
68021 COLMAR (FRANCE)

COMPARAISON DES SYMPTOMES ET TRANSMISSION PAR GREFFAGE D'UNE MOSAIQUE NERVAIRE DE *VITIS VINIFERA*, DE LA MARBRURE DE *V. RUPESTRIS* ET D'UNE AFFECTION NECROTIQUE DES NERVURES DE L'HYBRIDE *RUP.-BERL.* 110 R.

R. LEGIN et A. VUITTENEZ

En France, diverses altérations de la coloration verte des feuilles ont été reconnues indépendantes de la maladie du « Court-noué » parce qu'il n'est pas possible de mettre en évidence, chez les plantes atteintes, par test sérologique, un virus de type NEPO tel quel « fanleaf » ou « arabis mosaïc ». D'autre part, l'inoculation mécanique de plantes test herbacées, telles que les chénopodes, ne donne pas de résultats (VUITTENEZ, 1966).

Les expériences entreprises depuis montrent qu'il s'agit néanmoins de maladies de type viral, transmissibles aisément et de façon permanente par greffage à différentes espèces et variétés de Vigne. Les symptômes obtenus, lors de ces essais, permettent de distinguer trois types de maladies apparemment indépendantes:

I. - *Mosaïque des nervures*: Il s'agit typiquement d'une décoloration vert-clair, affectant toujours des plages assez étendues de

la surface foliaire, de localisation variable, mais bordant souvent les nervures principales et moyennes (vein-clearing). Dans d'autres cas la décoloration intéresse au contraire l'espace internervaire, laissant la coloration verte normale au voisinage des nervures (vein-banding). On trouve enfin, sur d'autres feuilles, des taches décolorées éparses formant une mosaïque en tout point semblable à celle du « Court-noué ». Sur les feuilles devenant adultes, dans les plages les plus atteintes par les décolorations, il peut se former des points nécrotiques bruns qui, en devenant confluents, aboutissent au dessèchement d'une partie de la feuille. Les points nécrotiques forment fréquemment un liseré le long des nervures mais ces dernières ne deviennent pas elles mêmes nécrotiques, contrairement à la maladie qui sera décrite au paragraphe 3. Parmi les variétés inoculées expérimentalement par greffage, *Vitis riparia* (Riparia gloire) manifeste les symptômes précédents avec beaucoup d'intensité (Fig. 1) et peut constituer un bon indicateur de la maladie.

Des expériences d'inoculation systématiques par greffage ont montré que la mosaïque des nervures est une affection commune, produisant des symptômes plus ou moins nets selon les périodes de la saison aussi bien chez *Vitis vinifera* (Syrah, Servant, Viognier, Chardonnay, Alphonse Lavallée, Muscat de Hambourg), que chez divers autres *Vitis* ou hybrides interspécifiques. Certaines vignes utilisées comme inoculums pour l'étude d'autres maladies, en particulier la maladie des cannelures du tronc (Legin, 1972) analogue au « Legno-riccio », ont transmis également des symptômes typiques de mosaïque des nervures à *V. riparia* ainsi qu'à *riparia-Berlandieri* Kober 5 BB, ce qui ne signifie pas pour autant que les deux catégories de symptômes correspondent à une même maladie. Il en est de même pour certains symptômes, apparemment identiques à ceux de la mosaïque des nervures, obtenus dans d'autres pays, en particulier la maladie décrite en Suisse sur Kober 5 BB et 5 C inoculés à partir de Chasselas infectés de façon latente (Bovey, 1970).

II. - *Marbrure* : Des observations préliminaires — déjà discutées lors de la réunion de l'ICVG à Nyon (Suisse) en 1964 — avaient montré que des lignées de *Vitis rupestris* multipliées en pépinière à Colmar par bouturage à partir de souches individuelles, se différenciaient selon leur origine par la vigueur, le port et l'aspect mosaïqué du feuillage par rapport à d'autres provenances d'aspect

normal de la même variété. En observant par transparence les feuilles de *rupestris* atteint, on constate que la mosaïque est constituée par des décolorations affectant toujours les plus fines nervures (Fig. 2); elles peuvent être néanmoins très nombreuses donnant aux espaces situés entre les nervures principales un aspect plus clair, jaune huileux, et conduisant à une distortion de la feuille.

2

3

L'absence de cordons endovasculaires chez les vignes atteintes, ainsi que l'impossibilité d'obtenir la transmission de virus par inoculation mécanique de plantes test herbacées ont conduit à donner la désignation spéciale de « marbrure » à cette maladie, pour la distinguer définitivement de la mosaïque liée au Courtnoué.

Les expériences d'inoculation par greffage de *V. rupestris* à partir de très nombreuses origines de vignes ont montré que l'agent responsable de la marbrure est extrèmement répandu, même chez les vignes sélectionnées sur le plan cultural aussi bien chez les porte-greffe que chez les vinifera. Lors des premières observations nous pensions que la marbrure obtenue sur *rupestris* et la mosaïque des nervures observée chez des *vinifera* pouvaient correspondre à une même maladie. En réalité, un nombre plus grand d'expériences et les travaux entrepris en particulier aux Etats-Unis sur la maladie du « Fleck », symptomatologiquement identique à la marbrure, ont montré que l'agent infectieux responsable est toujours hébergé de façon latente par les *vinifera* et de nombreuses autres espèces de vignes (Hewitt *et al.*, 1970). Il reste encore des incertitudes sur les symptômes constituant la réponse des diverses espèces de vignes à la marbrure. Ainsi, dans les expériences américaines, Kober 5 BB est considéré comme infecté avec symptômes; de même pour la maladie issue de chasselas décrite en Suisse, la réaction du *rupestris* est de type marbrure et des symptômes très intenses sont produits chez Kober 5 BB et 5 C. Nous avons relevé plus haut la similitude de ces derniers symptômes avec ceux obtenus dans les inoculations de la mosaïque des nervures. On peut supposer que dans les expériences précédentes, les inoculums pouvaient contenir à la fois à la fois marbrure et mosaïque des nervures. En effet lors de nos propres expériences d'inoculation par greffage d'une origine trés caractéristique de marbrure, seules les deux espèces *V. rupestris* et *rupestris-Berlandieri* 99 R ont donné une réaction très caractéristique utilisable pour l'indexage. Par contre les *riparia-Berlandieri*, tel Kober 5 BB ou *V. riparia* ne montraient absolument aucun symptôme.

III. - *Nécrose des nervures*: Une troisième affection à été détectée, initialement au cours d'expériences d'inoculation par greffage entre différentes espèces de vignes pour la recherche de variétés indicatrices de la marbrure et de l'enroulement. Les résultats ont montré qu'une autre maladie, présente aussi bien dans des

inoculums atteints de barbrure ou d'enroulement que dans des vignes exemptes de ces deux maladies, se transmettait par greffage en induisant des symptômes nécrotiques particulièrement nets sur la variété d'hybride porte-greffe *rupestris-Berlandieri* 110 R (Fig.3) : dès l'année de l'inoculation, on note une importante réduction de croissance chez les plantes où l'infection a réussi, puis l'apparition de nécroses noires des nervures commençant sur les feuilles basales des pousses. Au fur et à mesure du développement des vignes, le nombre des feuilles atteintes augmente, les nécroses intéressent un nombre de plus en plus grand de nervures formant un réticulum noir, particulièrement visible à la face inférieure des feuilles qui finissent par se dessécher partiellement avant la chute normale en fin de végétation. On peut noter aussi, sur certaines pousses des 110 R infectés, la présence de stries noires longitudinales (streak). *Vitis vulpina* peut présenter également des symptômes de même nature.

Les résultats d'indexage entrepris avec la variété 110 R sur une gamme étendue d'inoculums, en particulier des clones d'aspect normal déjà sélectionné sur le plan cultural, ont montré que l'agent de la nécrose des nervures est communément répandu. De nouvelles expériences devront préciser les rapports de cette maladie avec la mosaïque des nervures et établir si ces diverses affections semi-latentes de la vigne ont des effets sur le plan cultural.

SUMMARY

Three types of disorders producing different degrees of mosaic and necrosis on leaves of the grapevine are described. Experiments of inoculation by grafting have established that these conditions are readily transmitted. Several grapevine varieties, producing strong symptoms, can be used as indicators for indexing purposes.

The comparison of the results obtained from a large number of inoculums of grapevines, previously indexed for known diseases shows no relation of these symptoms with « Nepo »-viruses or leafroll.

The three types of symptoms prooved to be apparently independent, corresponding to different diseases for which the following names and synonymes are tentatively proposed :

— *Vein mosaic*: (syn. vein clearing, Adernbanderung, mosaique des nervures), producing more or less conspicuous symptoms in *vinifera* or other *Vitis* sp. Best indicator: *Vitis riparia*.

— *Small-vein mosaic*: (syn. fleck, marbrure), producing no symptoms in *vinifera* and many other varieties. Best indicator: *Vitis rupestris*.

— *Vein necrosis*: latent in many species of Vine. Best indicator: *rupestris-Berlandieri* 110 R.

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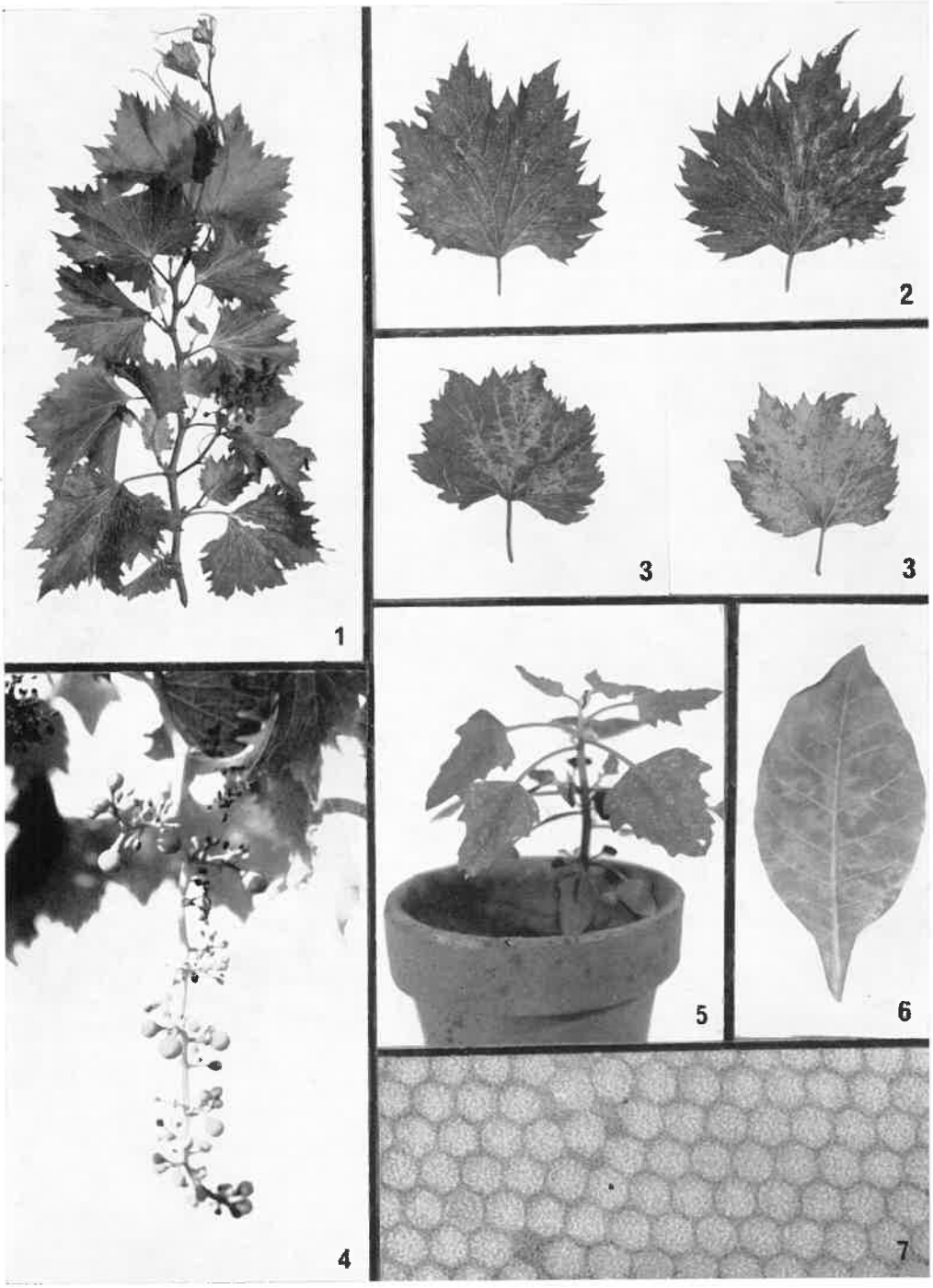
CDA RESEARCH STATION
VINELAND STATION, ONTARIO, CANADA

A SAP-TRANSMISSIBLE VIRUS ASSOCIATED WITH A SEVERE DISEASE OF THE HYBRID *VITIS* sp. cv JOANNES-SEYVE (26-205)

H.F. DIAS

An apparently new undescribed grapevine virus was found associated with an economically important disease of Joannes-Seyve (26-205) grapes in Ontario, Canada. Infected vines were severely stunted; leaves were mottled with line patterns and feathery veins (Fig. 1, 2). Sometimes the veins were light green or exhibited chromo yellow banding (Fig. 3). Often leaves were asymetrical with prominent teeth and an open petiole that resembles fanleaf symptoms (Fig. 2). Flower clusters tend to wither and dry up and the vines fail to set fruit (Fig. 4). The disease was widespread in one vineyard and its distribution pattern suggests that it might be soil-borne.

The virus was readily transmitted to *Chenopodium quinoa* Willd., *Cucumis sativus* L. cv 'Windermoor Wonder', *Nicotiana tabacum* L. cv 'Harrow Velvet' and *Gomphrena globosa* L. when inoculated with sap from infected grape leaves macerated in a 2.5% nicotine solution or 0.07 M TRIS. Chlorotic or necrotic lesions developed on inoculated leaves of *C. quinoa* within 5 days followed by a systemic mottle, distortion and necrosis of the shoot tip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(Fig. 5). In tobacco, local chlorotic lesions appeared in 7-10 days. In winter, chlorotic or necrotic ringspots usually develop. Systemic mottle on which chlorotic or necrotic rings were sometimes superimposed appeared later (Fig. 6). Plants usually recovered and in most cases were symptomless. In cucumber, primary chlorotic lesions occurred on the cotyledons after inoculation followed by a systemic mottle or blotching of the true leaves. In gomphrena, buff-centered lesions or chlorotic rings sometimes with reddish margines appeared in 5-8 days after inoculation.

The virus has a wide host range. Species of the *Chenopodiaceae* are extremely susceptible but the symptoms on thse are generally milder than in *C. quinoa*. Chlorotic lesions may or may not develop in the inoculated leaves of *Petunia hybrida* Vilm. cv 'Balcony', *Datura stramonium* L., *Lycopersicum esculentum* L., *N. glutinosa* L., *Phaseolus vulgaris* L. and *Vigna sinensis* L. Systemic symptoms in these species include blotching, vein clearing or mottle but they are usually mild and transient.

In crude *C. quinoa* sap, the thermal inactiviation point in 65-70 C., the dilution end point 10^{-6} - 10^{7} and the virus remains infectious in sap for about 12-15 days at room temperature. Little infective was lost from infectious sap stored at sub-zero temperatures for 6 months. The virus precipitates at pH 4.0. Seed of infected *C. quinoa* contained the virus.

Highly infective virus preparations were obtained by butanol-chloroform clarification and differential centrifugation. Purified preparations have 2 nucleo-protein components that sediment at different rates in sucrose gradients. Examination of preparations of the top (T) and bottom (B) components further purified by additional centrifugation in sucrose gradients indicates that both components are polyhedral particles of approximately 26 mμ diameter (Fig. 7). The UV absorbance ratios at 260/280 was 1.59 (T) and 1.61 (B) respectively. Both components are closely related serologically. Preliminary work suggests that both components are required to reproduce the disease; neither components were infectious when inoculated separately in *C. quinoa*. However, when mixed they reproduce the typical disease symptoms in this species.

The virus is not serologically related to grape fanleaf virus group, arabis mosaic, tomato ringspot, tobacco ringspot, tomato bushy stunt, raspberry ringspot, grapevine chromo mosaic or to several strains (type, elderberry, dogwood and rhubarb) of the

cherry leaf roll virus with which this new grape virus has some similarities.

The virus described herein appears to be new in grapes and, at present, seems restricted to the cv 'Joannes-Seyve' in a limited number of vineyards. Currently it is not known whether this virus was introduced into Ontario in imported grapes or is of native origin having been acquired by the cultivar from infectious soils in the Niagara Peninsula.

RIASSUNTO

Un virus trasmissibile per succo, associato con una grave malattia della vite Joannes-Seyve (26-205)

Un virus apparentemente non ancora descritto è stato isolato da viti cv. Joannes-Seyve (26-205) affette da una malattia economicamente importante nell'Ontario (Canada). Sono descritti i sintomi riscontrati sulle viti malate.

Il virus può essere trasmesso per succo a varie piante erbacee, tra cui *Chenopodium quinoa, Cucumis sativus, Nicotiana tabacum, Gomphrena globosa*. Il virus risulta costituito da due componenti (« top » e « bottom »), entrambi poliedrici e aventi diametro pari a 26 mμ. Non è sierologicamente correlato con il virus dell'arricciamento (GFV) nè con altri virus del tipo « NEPO ».

Non si hanno notizie certe sulla provenienza della malattia.

INSTITUTE OF BIOLOGY AND PEDOLOGY, ACADEMY OF SCIENCES OF THE USSR, VLADIVOSTOK;
UKRAINIAN RESEARCH INSTITUTE FOR VITICULTURE AND WINE-MAKING, ODESSA

A GRAPEVINE VIRUS DISEASE IN PRIMORYE TERRITORY, USSR.

I.N. SAMONINA, B.N. MILKUS, A.V. KRYLOV and N.V. KRYLOVA

Observations of vineyards over Primorye Territory in 1971 showed virus-like symptoms with var. Alfa. The symptoms resembled strongly pronounced light-green designs, usually of oak-like patterns formed by single or numerous irregular lines or stripes (fig. 1). The linear patterns were similar to those induced by plum, peach and cherry linear pattern viruses (CADMAN *et al.*, 1958). At times, leaf deformation was observed. The symptoms appeared at the beginning of July and persisted throughout the season. Affected plants were distinguished by retarted growth.

MATERIAL AND METHODS

Mechanical grapevine-virus inoculation to herbaceous test-plants was carried out in December by means of squeezed-out sap. Two or three eye cuttings from affected plants were preliminarily planted in a green-house; test-plants were grown here too at +20 to 22°C. Leaves ground in a mortar with 0.1 M tris-buffer (pH 8.0), 1% caffeine, 0.2% ascorbic acid and 0.2% sodium sulphite were

used to prepare the inoculum. The leaves (1 g) were ground in the buffer (4 ml). The indicator-plant leaves dusted with carborundum were rubbed with the inoculum and subsequently washed with water.

Leaves with strongly pronounced symptoms were used to make preparations for electron microscopy.

The virus was purified by two differential centrifugation cycles and concentrated with polyethylene glycol (mol. wt. 6,000). The partly purified preparation was placed on grids, contrasted by phosphotungstic acid (pH 6.0) and examined in a « Hitachi-7 » electron microscope. Detection of intracellular virus inclusions was

carried out by the CRISTIE method (1967). The sections were fixed with 2.5% thrichloracetic acid and subsequently coloured with 1% calcomynorange with lucol brilliant green during 5 minutes.

RESULTS

The investigation results showed that the virus is not transferred mechanically (see Table 1). Thus, none of the test-plants reacted to inoculation.

Electronic microscopy showed rod-shaped virus particles. Spherical particles, whose presence in the sap was expected to be due to symptoms with strongly-pronounced ringspot, were not detected. Apparently, their concentration is low or they are unevenly distributed in vine plants. As for the rod-shaped particles, their presence in the sap indicated a mixed infection.

Crystalline inclusions were found in the epidermal tissues of the stem, petioles and in the hairs. The treatment of the inclusions with acrydinorange caused characteristic of RNA red fluorescence.

TABLE 1.

Results of mechanical transfer of « Grapevine linear pattern virus » on herbaceous indicators

Indicator-plant	Inoculation results: numerator = number of inoculated plants; denominator = number of plants with disease symptoms
1. *Chenopodium quinoa* Willd.	30/0
2. *Ch. amaranticolor* Coste and Reyn	30/0
3. *Ch. murale* L.	30/0
4. *Cucumis sativus* L.	10/0
5. *Petunia hybrida* Vilm.	10/0

DISCUSSION

During our investigations in Primorye Territory, a virus disease was detected, whose external symptoms resembled those of « the grapevine linear pattern virus ». However, we failed to transfer the virus mechanically on herbaceous test-plants. In partly purified preparations, rod-shaped particles were detected.

In grapevine affected by the infectious chlorosis virus, inclusions were detected by SCHTERENBERG, MILKUS and KUSHPELIOVA (1968). However, these inclusions were rare, and the method could not be used to diagnose the disease. « Grapevine linear pattern virus induces a multitude of crystalline inclusions in the plant tissues, thus giving further evidence of the virus. Hence, this method is quite applicable for diagnosing the disease.

Ostensibly the virus spreads with planting material, since numerous diseased plants were detected in the Nursery State Farm « Vinogradarsky » and at consumer grapevine-farms.

The following investigations are planned for the future:

1. Virus transfer on indicator stocks by means of green grafting.
2. Virus purification and antiserum preparation.
3. Determination of latent viruses.
4. Observations on the growth of the affected vines and disease distribution.

SUMMARY

A virus-like grapevine disease was detected in Primorye Territory, USSR. Rod-shaped particles were found by examining partially purified preparations in an electron microscope. Crystalline inclusions were found in the epidermal tissues of the stem, petioles and hairs. The disease is apparently induced by the « grapevine linear pattern virus ».

RIASSUNTO

Una virosi della vite in Primorje (U.R.S.S.)

Una malattia della vite riferibile a virus è stata osservata in Primorje (U.R.S.S.). In preparati purificati, esaminati al microscopio elettronico, sono state riscontrate particelle a forma di bastoncino. Inclusioni cristalline sono risultate presenti nei tessuti epidermici di germogli, di piccioli e peli fogliari. La malattia viene attribuita ad un virus indicato come « grapevine linear pattern virus » (virus della maculatura lineare della vite).

ISTITUTO DI PATOLOGIA VEGETALE, UNIVERSITÀ, BOLOGNA

VIRUS X DELLA PATATA ISOLATO DA RADICI E FOGLIE DI VITE

L. GIUNCHEDI

In questa nota riferiamo su un virus isolato meccanicamente, nel corso dei saggi sulle rivelatrici erbacee, da una pianta di vite cv « Barbera » ottenuta dall'apice vegetativo di un germoglio dopo 100 gg. di permanenza in camera di termoterapia a + 37°C. La pianta non manifestava nessun sintomo evidente e presentava una crescita normale. Il virus in esame è stato isolato anche dalla pianta madre dopo il trattamento termico. Quest'ultima presentava leggeri sintomi di accartocciamento.

MATERIALI E METODI

Il virus in esame è stato trasmesso inoculando le piante di saggio previamente cosparse di carborundum con il succo ottenuto triturando piccole porzioni di radici o giovani foglie in tampone solfito 0,01 M pH 7. Le proprietà fisiche in vitro sono state determinate secondo le tecniche convenzionali usando succo di *Nicotiana glutinosa* e *Gomphrena globosa* addizionato 1 a 1 con tampone fosfato 0,01 M pH 7 e adoperando le stesse specie per il saggio. Le prove di protezione incrociata sono state effettuate utilizzando

gruppi di 5 piante di *N. glutinosa* inoculate dapprima con il nostro virus e con il ceppo PV 54 del PVX poi, alla comparsa dei sintomi sistemici, rispettivamente con il ceppo PV 54 del PVX e con quello in esame (1). Comtemporaneamente all'ultimo inoculo come controllo venivano infettate 5 piante sane per ogni isolato.

La purificazione del virus è stata effettuata da foglie di *N. glutinosa* raccolte 15-18 giorni dopo l'inoculo e conservate in congelatore per circa 2 settimane. Il metodo seguito è quello descritto da Francki e McLean (1968) basato sull'impiego di carbone vegetale, Deae-cellulosa, celite, cloroformio e cicli di centrifugazione differenziale. Le sospensioni virali così purificate sono state inoculate su *Chenopodium amaranticolor* per la prova dell'infettività, usate per la lettura allo spettrofotometro, per l'osservazione microscopica e per preparare l'antisiero.

Le osservazioni al microscopio elettronico sono state effettuate con sospensioni purificate montate in PTA neutro al 2% e con preparati ottenuti dalle foglie di *C. amaranticolor* ombreggiati al cromo. Contemporaneamente sono state effettuate come controllo osservazioni sul mosaico del tabacco. La lunghezza normale delle particelle è stata calcolata secondo la tecnica di Brandes e Wetter (1959).

L'antisiero contro il virus in esame è stato preparato iniettando ad un coniglio per via intramuscolare 2,5 cc di sospensione purificata di virus omogeneizzata con un uguale volume di adiuvante di Freud. Le iniezioni sono state fatte in numero di 5 ad intervalli settimanali.

La titolazione dell'antisiero, effettuata 15 giorni dopo l'ultima iniezione e le altre prove sierologiche sono state eseguite con la tecnica della precipitazione in tubo.

Le prove di trasmissione meccanica del virus purificato a vite sono state effettuate inoculando semenzali di « Barbera » allevati in terreno sterile e tenuti al buio 48 ore prima dell'inoculo allo stadio di 4-5 foglie. Prima dell'inoculo le piante sono state saggiate su *C. amaranticolor* e *N. glutinosa* per accertare la presenza di virus da seme.

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(1) Il ceppo PV 54 del PVX e il siero immune a questo virus ci sono stati gentilmente forniti dalla American Type Culture Collection, Rockville, Maryland, U.S.A.

I saggi sulle viti indicatrici sono stati effettuati mediante la inserzione di tessuti della vite ottenuta dalla porzione apicale del germoglio dopo il trattamento in camera di termoterapia su una serie di tre piante per ognuna delle seguenti indicatrici: St. George, Mission, LN-33.

RISULTATI

Inoculazione su ospiti

Il virus è stato trasmesso facilmente a *C. amaranticolor* e *G. globosa* da giovani radici mentre il suo isolamento da foglie è stato possibile solo all'inizio della vegetazione.

Qui di seguito sono descritti i sintomi osservati sugli ospiti più caratteristici, inoculati partendo da una singola lesione di *C. amaranticolor* o *G. globosa*, per la identificazione del virus. *Solanum demissum* « A_6 »: reagisce con lesioni locali necrotiche e macchie irregolari di colore verde chiaro nelle foglie della cima. *Gonphrena globosa* L.: sulle foglie inoculate si manifestano 8-10 gg. dopo l'infezione macchie necrotiche anulari di 2-3 mm di diametro circondate da un alone rossastro.

Nicotiana glutinosa: reagisce con anelli necrotici concentrici sulle foglie inoculate e macchie clorotiche evidenti sulle foglie di nuova formazione.

Nei saggi di premunità le piante infette con il virus in esame e con il ceppo PV 54 del PVX non hanno manifestato alcun sintomo locale quando reinoculate rispettivamente con l'isolato PV 54 e con il nostro virus; i due virus hanno causato nei controlli sintomi tipici. I semenzali di vite « Barbera » inoculati con la sospensione purificata infettiva non hanno manifestato sintomi evidenti e apprezzabili differenze di sviluppo rispetto ai controlli. Nessun sintomo è stato osservato dopo un anno dalla inoculazione anche sulle piante indicatrici del genere *Vitis*.

Proprietà fisiche e sierologiche

Il grado di inattivazione termica è risultato compreso fra 65-70°C; il punto di diluizione limite tra 10^{-5}-10^{-6}; la longevità in vitro, alla temperatura di 22-24°C, oltre 1 mese. Il metodo di puri-

ficazione adottato ci ha permesso di ottenere sospensioni virali altamente infettive senza apprezzabili impurità al microscopio elettronico e con uno spettro di assorbimento in luce ultravioletta tipico di una nucleoproteina con i seguenti rapporti caratteristici:

$$E' \max_{(262)}/E' \min_{(246)} = 1{,}25 \qquad b'v = 0{,}80 \qquad bv = 0{,}80$$

$$E \max_{(264)}/E \min_{(244)} = 1{,}39$$

Nelle prove sierologiche il siero immune al nostro isolato ha reagito con l'antigene omologo fino alla diluizione limite 1:1024 mentre non ha reagito contro il succo grezzo o chiarificato di *N. glutinosa*. Il virus purificato ha reagito chiaramente con l'antisiero immune al PVX-PV54. Le osservazioni al microscopio elettronico delle sospensioni purificate e dei preparati ottenuti dai tessuti infetti ci hanno permesso di osservare particelle di virus allungate e flessuose. La lunghezza normale delle particelle misurate dai preparati ombreggiati al cromo è risultata di 517 nm, mentre quella del mosaico del tabacco preso come campione è stata di 304 nm.

CONCLUSIONI

Le reazioni sintomatologiche sulle piante ospiti erbacee, le prove di premunità, le proprietà fisiche e sierologiche indicano che il virus isolato da vite è un ceppo del virus X della patata. Sebbene il virus non sia stato reisolato dalle piante di vite inoculate meccanicamente il suo ripetuto isolamento da vite naturalmente infetta fa ritenere questa pianta una portatrice latente del virus X della patata. E' questa la prima volta che detto virus viene isolato da vite.

Le osservazioni da noi compiute sembrano indicare che il virus in esame non aggrava i sintomi prodotti da quello dell'accartocciamento e che 100 giorni di permanenza della pianta infetta in camera di termoterapia non sono sufficienti per ottenere apici vegetativi che ne siano esenti. Sono in corso prove per infettare giovani semenzali di vite mediante inserzione di tessuti di piante erbacee infette e per trasmettere il virus a vite mediante inoculazione meccanica delle radici.

RIASSUNTO

Un virus a particelle allungate è stato isolato meccanicamente da una pianta di vite cv « Barbera » ottenuta dall'apice vegetativo di un germoglio dopo 100 giorni di permanenza in camera di termoterapia a + 37°C e dalla pianta madre dopo il trattamento termico. La prima non manifestava nessun sintomo evidente mentre la pianta madre presentava leggeri sintomi di accartocciamento. Le reazioni sintomatologiche sulle piante ospiti erbacee, le prove di premunità, le proprietà fisiche e sierologiche indicano che il virus isolato da vite è un ceppo del virus X della patata.

Nessun sintomo è stato osservato dopo un anno dalla inoculazione su piante indicatrici del genere *Vitis* e il virus non è stato reisolato da piante di vite inoculate meccanicamente.

SUMMARY

Potato Virus X isolated from grapevine roots and leaves

A virus with elonged particles has been mechanically isolated from grapevine plant cv « Barbera », obtained from the vegetative apex of a shot after 100 days of permanence in a thermotherapy room at + 37°C and from the mother plant after the thermic treatment. The former did not show any symptom, whilst the mother plant showed light symptoms of leaf-roll.

The symptomatological reactions on the herbaceous host plants, the pre-immunity tests, the physic and serologic properties, point out that the virus isolated from vine plant is a strain of potato virus X.

No symptom has been observed after one year from graft indexing on grape indicators, and the virus has not been reisolated from grapevine plants mechanically inoculated.

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SESSIONE II

VETTORI E RELAZIONI OSPITE-VETTORE

Vectors and host-vector relationships

(*chairman*: H. Dias)

BIOL. BUNDESANSTALT, MÜNSTER, GERMANY

RECENT STUDIES ON NEMATODES TRANSMITTING GRAPEVINE VIRUSES

B. WEISCHER

Recently HEWITT (1970) surveyed the present state of knowledge of grapevine viruses and in 1972 the bibliography by CAUDWELL *et al.* was published. In the present review primary attention is given to those publications not included in these bibliographies.

At present some 30 different viruses have been isolated and identified from grapes, of which seven can be transmitted by nematodes (Tab. 1). Although not all these succesful transmission experiments were performed with virus isolates from grapes, it seemed justified to include them in order to give a more complete survey. It must, however be kept in mind that a nematode species may not be able to transmit all strains of a virus as will be discussed later on. As the table shows, only polyhedral viruses transmitted by species of *Xiphinema* and *Longidorus* have been isolated from grapes. Although species of *Trichodorus* have sporadically been found in vineyards, no nematode transmissible tubular viruses have been detected in grapes so far. Most of the virus-vector relations mentioned in the table have been known for a number of years. Recently VALDEZ (1972 a) reported the first succesful transmission of raspberry ringspot virus by *Longidorus caespiticola, L. leptocephalus* and *Xiphinema diversicaudatum*. *L. caespiticola*

also transmitted arabis mosaic virus. This is the first record of a virus transmission by *L. caespiticola* and *L. leptocephalus*. Earlier attempts to transmit tomato blackring virus by *L. caespiticola* failed (Van Hoof, 1966). The recent successes may at least partly be due to the more refined method developed by Valdez (1972 b), who used microcontainers kept in moist chambers.

Table 1

Nematode vectors occurring in vineyards and the viruses they transmit

Nematode species	
Xiphinema americanum . . .	tomato ringspot peach rosette mosaic tobacco ringspot
Xiphinema diversicaudatum . .	arabis mosaic raspberry ringspot
Xiphinema index	grapevine fanleaf
Xiphinema italiae	grapevine fanleaf
Langidorus attenuatus . . .	tomato blackring
Longidorus caespiticola . . .	arabis mosaic raspberry ringspot
Longidorus elongatus	raspberry ringspot tomato blackring
Longidorus macrosoma . . .	raspberry ringspot

There are several records of « close associations » between *X. vuittenezi* and grapevine viruses. This species is the most common and often the only suspected vector in vineyards in Germany, but so far all attempts to transmit one of the grapevine viruses by it have failed.

At present 59 valid species of *Xiphinema* have been described. Of these 11 are known to occur in European vineyards (Weischer in press). In their revision Cohn et Sher (1972) erected 8 new subgenera.

Whereas the group of *X. diversicaudatum* (= subgenus *Diversiphinema*) is reasonably well analyzed, the *X. americanum*-group still offers some difficulties. What has been called *X. americanum* for a long time and is still called so, particularly by non-nematologists, proved to be a complex of morphologically closely related species. The corresponding subgenus *Xiphinema* comprises *X. americanum*, *X. brevicolle*, *X. mediterraneum*, *X. opisthohysterum* and *X. rivesi*. Since the geographical distribution of these species is incompletely known it is possible that some of the transmission experiments thought to be carried out with *X. americanum* were in fact done with other similar species. *Longidorus* is less well understood than *Xiphinema* but this may change in the near future. In 1963 Siddiqi *et al.* separated *Paralongidorus* from *Longidorus*. At present 60 species belonging to the two genera are known, 37 to *Longidorus* and 23 to *Paralongidorus*. Abul-Eid (1970) made a thorough comparative study and found the width of the amphidial aperture to be the only decisive differential character. Virus vectors are known only from *Longidorus* and not from *Paralongidorus*.

The geographical distribution and the ecological factors governing the occurrence of virus-transmitting nematodes in vineyards are still far from being well understood. Recently Dalmasso (1970) studied the influence of some ecological factors on the biological activity and the distribution of Longidoridae in France. He found the climate was the most important factor because it also influences the effects of plants and soil on nematodes. He distinguished four ecological groups: atlantic species (e.g. *X. diversicaudatum*, *L. macrosoma*), continental species (e.g. *X. vuittenezi*), mediterranean species (e.g. *X. italiae*, *X. mediterraneum*) and recently introduced species (e.g. *X. brevicolle*, *L. taniucha*). Prota *et al.* (1970) and Lamberti *et al.* (1972) studied the distribution of Longidoridae in Italy and observed a surprising irregularity of occurrence and predominancy in the different regions.

It must, however, be noted that our methods of extraction of nematodes from soil are very crude and that nematodes may well be present despite the negative results from examination of soil samples.

During the author's work in Germany in a number of cases where the first attempts to find a suitable vector for an obvious soil transmission of grapevine viruses failed, vectors could be

found by a more detailed sampling of the vineyard. Consequently it was found that *X. index* is more common in German vineyards than was reported earlier by Weischer (1966). Its occurrence in Germany is always connected with the presence of the so called « Reisigkrankheit », a complex virus disease. In Italy Palmisano (1970) found *X. index* only in 16.6% of the vineyards showing virus symptoms. In addition to errors in extraction, methods of soil sampling may also introduce considerable errors, mainly due to the irregular distribution of nematodes in the soil. Recently D'Errico (1970) and Tobar Jimenez *et al.* (1970) made detailed studies on the distribution of *Xiphinema* spp. in vineyards. Nematodes were found only in the immediate vicinity of living roots. They occurred to 1m depth (maximum depth analyzed) with the highest population density around 35cm. In California vineyards *X. index* occurred to a depth of 3.6m (Raski *et al.*, 1965). *Longidorus macrosoma* increased with depth up to 70cm and only then decreased (Flegg, 1968 a). Tobar *et al.* (1970) showed that also the horizontal distribution varies according to the species.

The observed population densities of nematodes transmitting grapevine viruses in vineyards are generally low, for the majority of species less than ten and rarely more than 20 individuals per 250ml of soil. This may partly be due to poor techniques but probably it reflects the true state of affairs. Flegg (1968 b) studied the lifecycle of some *Xiphinema* and *Longidorus* species and found a brief period of egg production only in early summer. The development from egg to adult took at least 2 years for *X. diversicaudatum* and *X. vuittenezi* (and very probably for others too) and the life span seems commonly to be between 3 and 5 years. Limited propagation period and slow development may well result in the low natural densities observed. *Xiphinema index* (Radewald & Raski, 1962) and *X. americanum* (Flores & Chapman, 1965) needed less than 30 days to complete their life-cycles under optimum conditions, which, of course, are seldom present in the field. Generally the population increase is more rapid with species multiplying parthenogenetically than in bisexual species multiplying amphimictically.

The available data on the specificity of virus-vector relations are still somewhat contradictory as they were from the very beginning. Reliable experimental evidence shows that in some cases a nematode species can transmit one single strain of a virus only and that even populations of the same species may vary in their

vector abilities, thus demonstrating highly specific relations between virus and vector (VAN HOOF, 1966; AYALA & ALLEN, 1966). There is, however, an increasing number of reports indicating that one species may be able to transmit several viruses, and that one virus may be transmitted by several nematode species. Based on this recent information the author is inclined to believe that multiple transmission is the rule rather than the exception.

The actual location of virus particles in the vector nematodes was unknown until recently. TAYLOR & ROBERTSON (1969, 1970 a, 1970 b) obtained precise information by careful electronmicroscopic analysis. In *Longidorus elongatus* which had fed on plants infested with tomato blackring and raspberry ringspot viruses, the virus particles were found in the buccal capsule and in the space between the stylet and the guiding sheath. When they fed on plants infested with arabis mosaic virus, which is not transmitted by this species, the nematodes carried a few particles only in the buccal capsule but none in the guiding sheath. This suggests that the association of virus particles with the cuticular lining of the guiding sheath is responsible for the specificity of virus transmission in *Longidorus*. In *Xiphinema* species the particles were retained as a monolayer absorbed on to the cuticle lining the lumina of the odontophore, anterior oesophagus and oesophageal bulb; but none were found in the lumen of the odontostyl. In *Trichodorus pachydermus* particles of tobacco rattle virus were similarly attached to the cuticular lining of the oesophagus. After feeding on plants infested with a virus they do not transmit, the *Xiphinema* and *Trichidorus* species tested had none or very few particles in the oesophagus. This indicates that the virus-vector relations are associated with the adsorption of virus particles on to the oesophageal cuticle. Obviously all nematode species feeding on virus infected plants ingest virus particles, but the particles are retained in the oesophageal region only by vectors, and are then transmitted during the next feeding process. In non-vectors the particles pass with the sap directly through the oesophagus to the intestine and are lost for an active transmission. The sites of virus retention in *Xiphinema* provide an explanation for the observation that infectivity is not retained through the moult. During the moulting process the cuticular lining of the oesophagus and of the odontophore is shed and together with the virus particles ingested into the intestine. Recently VALDEZ (1972) succesfully transmitted a strain of rasp-

berry ringspot virus by *X. diversicaudatum,* although Taylor & Robertson (1970 a) found no particles in the oesophagus of this species following feeding on plants infested with the virus, and concluded that *X. diversicaudatum* was not a vector for raspberry ringspot virus.

It has been known from early reports that the nematode vectors have different retention periods. Species of *Xiphinema* can remain infective for 10 months, whereas *Longidorus* lose their infectivity within some weeks. The differences in virus location in their respective vectors may account for this. The figures mentioned were observed in individuals. As for populations the situation is different. Populations of *X. index* carrying grapevine fanleaf virus maintained infectivity for more than 8 months in host free soil, but the percentage of infective nematodes within this population decreased sharply after 6 weeks to some 10% (Taylor & Raski, 1964). All these results should be considered as preliminary. This was demonstrated by Van Hoof (1970) who showed that *Trichodorus*, for years known to remain infective for up to 10 months, transmitted tobacco rattle virus after a storage period of 3 years without any access to plants. Whether a nematode remains infective over a longer or a shorter period is often of little practical importance, since common weeds may harbour several viruses and offer chances for reinfection.

Little information is available on the biological effect of viruses on nematodes. Roggen (1966) reported slight physiological and anatomical alterations in *X. index* by grapevine fanleaf virus. Ayala & Allen (1966) showed that tobacco rattle virus increases the rate of reproduction of its vector *Trichodorus allius.* Das & Raski (1969) found that grapevine fanleaf virus significantly increased the rate of survival of viruliferous *X. index.* In studies on the effect of six different plant viruses on two non-vector species Weischer (1969, 1970) observed that some of these viruses inhibited the multiplication whereas others lead to higher populations as compared to populations living in virus-free plants. Although some observations suggest a direct influence of the virus on the physiology of the nematodes, most of the phenomena observed could also be an effect of the virus diseased plant on the nematode.

The present survey, summarizing important recent information about nematodes which transmit grapevine viruses, indicates the considerable volume of data obtained during the past 15 years since

the first record; but it indicates equally well the many questions still unanswered. As yet many experimental results are contradictory. In assessing the value of the available experimental evidence it must be recognized that techniques have varied and developed during the 15-year period. Consequently many of the data are not exactly comparable. The ability of nematodes to transmit a virus under experimental conditions can be affected by extraction technique, potting soil, size of test pots, age of plants, temperature etc. Furthermore the terminology and the experimental approaches still follow those used in studies of insect-transmitted viruses and this may well be a deterrent to nematological investigations.

RIASSUNTO

Recenti studi sui nematodi che trasmettono i virus della vite

Finora sono stati isolati dalla vite 30 diversi virus, 7 dei quali, tutti poliedrici, possono essere trasmessi da nematodi che appartengono ai generi *Xiphinema* e *Longidorus*.

E' riportata una recente segnalazione di due specie, *L. caespiticola* e *L. leptocephalus*, finora non conosciute come vettori di virus.

La distribuzione geografica e i fattori ecologici che regolano la presenza nei vigneti dei nematodi vettori di virus non sono ancora ben chiari, nonostante siano state fatte alcune ricerche in merito che sono riferite. Sono riportati alcuni dati relativi alla distribuzione nel terreno di alcune specie di nematodi, sia in senso verticale che orizzontale.

La densità della popolazione dei nematodi vettori di virus è generalmente bassa, meno di 10 e raramente più di 20 individui per 250 cc di terreno.

I dati sulla specificità della relazione tra virus e vettore sono contraddittori; l'opinione personale dell'Autore è che la trasmissione multipla sia la regola, piuttosto che l'eccezione.

Ricerche al microscopio elettronico indicano che le relazioni tra virus e vettore nel genere *Longidorus* sono legate all'associazione delle particelle di virus con il rivestimento cuticolare della guaina dello stiletto, e nel genere *Xiphinema* all'assorbimento del virus da parte della cuticola che riveste il lume dello stiletto, dell'esofago anteriore e del bulbo dell'esofago. Questo spiega perchè nel genere *Xiphinema* l'infettività è perduta con la muta, infatti durante questo processo i rivestimenti cuticolari dell'esofago e dello stiletto sono eliminati e digeriti dall'intestino.

La diversa lunghezza del periodo di ritenzione del virus in singoli individui dei generi *Longidorus* e *Xiphinema* può essere spiegata con la diversa localizzazione del virus. I dati relativi alla conservazione dell'infettività da parte di popolazioni di nematodi sono ancora in una fase preliminare.

Sono riportate alcune osservazioni sugli effetti biologici dei virus sui nematodi vettori.

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I.N.R.A. STATION DE RECHERCHES FORESTIÈRES
Avenue A. Vivaldi - 84000 AVIGNON - France

INSECTES VECTEURS DE MALADIES À VIRUS ET À MYCOPLASMES DE LA VIGNE

D. SCHVESTER

A l'exception de brèves indications dans la bibliographie rassemblée par Caudwell, Hewitt et Bovey (1972) les dernières revues générales sur la question des Insectes vecteurs de Maladies de la vigne, remontent à la réunion de Davis, en 1965, du Groupe d'Etudes.

Il n'y a eu entre temps, aucune identification nouvelle d'Insecte vecteur, et les deux seules maladies de la vigne formellement reconnues à ce jour, pour être transmisse par des Insectes demeurent encore :

I - La Maladie de Pierce, pour laquelle 24 espèces on été reconnues vectrices.

II - La Flavescence Dorée (F.D.), dont le seul vecteur naturel actuellement connu, demeure *Scaphoïdeus littoralis* Ball.

Les études sur la Maladie de Pierce et ses vecteurs n'ont fait l'objet d'aucune publication nouvelle. Nous proposons donc de restreindre cet exposé au problème des vecteurs de la F.D. et des maladies qui lui sont apparentées par les symptômes, c'est-à-dire, les Maladies de « type Jaunisse ».

I - LES « JAUNISSES » DE LA VIGNE

La plus connue et la mieux étudiée est la Flavescence Dorée « *sensu stricto* » c'est-à-dire la Maladie qui a sévi à l'état épidémique dans le Sud-Ouest de la France, en particulier en Armagnac. Elle est transmise, dans la nature, par *Scaphoïdeus littoralis* Ball espèce importée d'Amérique en Europe et, selon l'état actuel des connaissances, strictement inféodée à la vigne. Vidano (1966) qui a recherché l'espèce dans son aire d'origine, confirme d'ailleurs ses moeurs amphélophiles.

Il semble acquis maintenant que la F.D. est une Maladie à Mycoplasmes: des corpuscules à allure mycoplasmatique ont été identifiés dans les tissus du liber de plantes infestées, comme dans les organes d'Insectes vecteurs (Caudwell, Gianotti *et al.*, 1971). Il est probable, comme c'est le cas pour la plupart des agents de Maladies du type Jaunisse (Withcomb et Davis, 1970) que les microorganismes demeurent confinés au tissu du phloeme.

Le « Bois Noir » induit sur la vigne, des symptômes très analogues. La Maladie existe dans les vignobles de Bourgogne et du Jura où sa propagation ne revêt pas actuellement de caractère épidémique, sauf en quelques cas restreints. Elle fut considérée jusqu'à une date récente, comme une forme de la F.D. « *sensu stricto* ». Mais l'échec des essais de transmission de cette forme par *Scaphoïdeus littoralis* amène maintenant Caudwell *et al.* (1971) à la considérer comme une Maladie différente. C'est probablement à celle-ci, plus qu'à la F.D. proprement dite qu'il faudrait rapporter les Jaunisses observées en Allemagne et en Suisse. Selon certaines observation de Bovey (1972) la forme de Suisse pourrait être apparentée au « Corky bark ».

Des symptômes de F.D. « *sensu lato* » ont été observés d'autre part en Roumanie, en Israël, en Italie et au Chili. Il n'est pas possible actuellement de les relier à l'une ou l'autre forme.

Pour être complet, il faut rappeler que Caudwell a obtenu, sur vigne, les manifestations d'une Jaunisse distincte de la F.D. et du Bois noir, par le processus artificiel suivant: inoculation à la Fève, par des *Euscelis* trouvés dans la nature porteurs de corpuscules (dits particules φ), puis report sur la vigne, au moyen de *Scaphoïdeus littoralis*.

Le fait qu'*Euscelis* ait été trouvé naturellement porteur de particules, n'a, par lui-même, rien de surprenant: on sait en effet

que cette espèce peut-être vectrice de plusieurs maladies à Mycoplasmes, en particulier de la *Phyllodie* du treèfle. Que l'une de ces maladies ait pû être expérimentalement transmise à la vigne, par un système de « relais », n'implique pas qu'elle puisse l'être dans la nature. L'important, dans ces observations est surtout que, selon l'expression de Caudwell, elles mettent en évidence la « pluralité des Jaunisses de la vigne », qu'il faut désormais poser, en principe, au moins à titre d'hypothèse de travail. Le g. *Vitis* est probablement un mauvais discriminateur et, exception faite peut-être, de certaines variétés typiquement indicatrices, semblerait souvent réagir globalement, de façon analogue et peu distincte à l'infection par des agents de Jaunisse différents.

Dans ce domaine, la réussite de la transmission de la F.D. à la Fève par Caudwell constitue déjà un progrès important (*Vicia faba* réagit de façon très différente à la F.D. et à la jaunisse φ) et la culture systématique des mycoplasmes telle qu'elle est rapportée par Gianotti *et al.* (1972) pourrait probablement contribuer efficacement à la distinction des divers agents.

II - TRANSMISSION DE LA FLAVESCENCE DORÉE

2.1. *Transmission par Scaphoïdeus littoralis* Ball.

Les caractéristiques de la transmission de la F.D. de la vigne à la vigne, observées dans les conditions expérimentales sous cage de nylon, mais en plein champ sont les suivantes (Schvester, Carle, Moutous, 1969).

Période minimale d'acquisition : 7-8 jours
Période minimale de latence : 28 jours

soit un total del 35 jours au minimum auxquels s'ajoute une durée nécessaire d'inoculation sur la plante saine, assez courte (quelques jours).

Caudwell, dans ses expériences sous serre a pu réduire cette durée à 21 jours. Ceci confirmerait l'hypothèse d'un effet des conditions extérieures sur les modalités de la transmission (Schvester, 1966). Il semble probable que dans des conditions absolument naturelles, lorsqu'est exclu « l'effet de serre » produit par les cages, la transmission nécessite de plus long délais. Il serait d'ailleurs intèressant de vérifier si seule la période d'acquisition, ou seule la

période de latence, ou les deux, sont affectées par les variations des conditions.

Caudwell remarque d'autre part, que la transmission de la F.D. de la fève à la fève ou de la fève à la vigne par *S. littoralis* ne requiert que des durées plus brèves encore.

2.2. *Possibilités de transmission par d'autres espèces*

Caudwell *et al.* ont réussi la transmission de la F.D. à *Vicia faba* (à l'état de plantule) avec *S. littoralis*. La maladie à pû être ensuite transmise de la fève à la fève par *Euscelis plebejus* et par *Euscelidius* sp.. Ceci peut remettre en question la possibilité de la transmission de la F.D. par d'autres vecteurs que *Scaphoïdeus*. Cette possibilité n'est pas absolument inconcevable, car la spécificité des Insectes à l'égard des Mycoplasmes peut n'être pas absolument stricte: certaines maladies peuvent être transmises par des Insectes différents et inversement, une même espèce peut transmettre des maladies différentes.

Carle et Moutous (1967) ont effectué, avec 15 espèces d'Insectes suceurs, 143 tests en tout, groupant 1200 individus. Les tests étaient effectués dans les mêmes conditions expérimentales qu'avec *S. littoralis*, en plein champ, sous capuchons de nylon. Les périodes d'acquisition et d'inoculation étaient toutefois adaptées aux possibilités de survie sur la vigne, de chaque espèce. Nous examinerons le cas, surtout, de *E. plebejus* et celui de *Aphrodes bicinctus*.

2.2.1. *Euscelis plebejus*

L'espèce est extrèmement fréquente dans les vignobles et dans les environs. C'est toutefois, surtout, une espèce de plantes basses, des prés, dont la présence dans la vigne est à rapporter le plus souvent à l'existence d'adventices. Même si l'on peut admettre qu'elle puisse, accidentellement, se nourrir sur vigne, la possibilité d'une vie larvaire prolongée sur cette culture paraît exclue.

Expérimentalement, la survie de *E. plebejus* adulte sur les vignes saines, peut dépasser 20 jours, mais n'excède pas 8 jours sur des plantes atteintes de F.D..

La période d'acquisition minimale chez *Scaphoïdeus littoralis* dans nos expérimentations de plein champ était de 7 jours, mais la période de latence est d'au moins 28 jours. Si l'on se réfère à ces données, *E. plebejus* pourraît théoriquement acquérir la maladie,

mais la période de latence est sensiblement supérieure à ses possibilités de survie sur la vigne. La transmission ne pourraît être réalisée que si l'Insecte, devenu infectieux, retournait un certain temps sur un végétal plus favorable à sa survie avant de se reporter sur vigne.

Une telle assimilation directe de *E. plebejus* à *S. littoralis* en ce qui concerne les caractéristiques de la transmission, n'est sans doute pas possible. Cependant la réunion des conditions nécessaires à une transmission dans la nature par *E. plebejus*, paraît extrêmement aléatoire. En fait les tests de vigne à vigne avec des durées d'acquisition de 60, 90 et 120 heures et des durées d'infection de plantes saines de 20 jours, ont été négatifs.

Il reste que la transmission paraît expérimentalement possible, directement de la fève à la vigne avec *E. plebejus* : si des expériences ont été faites sur ce point, les résultats n'en sont pas encore connus. Toutefois, la F.D. semble bien être une maladie dont l'agent est localisé au phloème (Caudwell, Gianotti *et al.*, 1971). Or si les piqûres de *E. plebejus* présentent un taux relativement élevé de terminaisons dans le phloème, sur pétioles de tomate et de trèfle, (Moreau communication personnelle, cf. Schvester, 1965) il semble au contraire que sur vigne, l'espèce soit surtout piqueur de xylème (Carle et Moutous, 1965). Il serait intéressant de vérifier d'une part la localisation des agents de la F.D. chez la Fève, d'autre part, le mode de nutrition sur cette plante, de *E. plebejus*.

2.2.2. *Aphrodes bicinctus*

C'est également une espèce des plantes basses, assez fréquente dans les vignes et vectrice potentielle de maladies à Mycoplasmes, en particulier du Stolbur.

La survie d'*Aphrodes bicinctus* sur vigne est un peu meilleure que celle d'*E. plebejus* et permet d'observer des périodes d'acquisition de 5 à 10 jours. La précaution fut prise cependant de maintenir les Insectes présumés infectés pendant 30 jours sur Trèfle, afin d'assurer la période de latence avant report sur vigne saine durant 20 jours. C'est par un artifice un peu analogue, en maintenant *Macropsis fuscula* Zett. sur *Rubus* un temps suffisant, pour que s'écoule la période de latence, que Van Der Meer et De Fluiter parviennent à transmettre le « *Rubus stunt* » au fraisier, sur lequel la Cicadelle ne vit, et ne se développe pas normalement.

Cependant, les 19 tests effectués avec *Aphrodes bicinctus* on été négatifs.

La conclusion est que *S. littoralis* est bien probablement le seul vecteur actuellement connu de la F.D. capable d'assurer une propagation épidémique de la maladie.

Jusqu'à présent, les autres hôtes végétaux connus de la maladie (*Vicia faba, Chrysanthémum carinatum*) ou ses autres vecteurs (*Euscelis plebejus, Euscelidius variegatus*) ne présentent d'intérêt qu'expérimental. Toutefois ces données nouvelles mettent en évidence le fait que la « spécificité pratique » de *S. littoralis* dans la transmission de la F.D. est probablement beaucoup plus liée à la spécificité de l'Insecte pour son hôte végétal, qu'à celle de l'agent infectieux pour la vigne et (ou) pour l'Insecte.

Elles renforcent l'hypothèse que d'autres espèces végétales que la vigne puissent constituer des foyers, peut-être latents, de l'agent infectieux: *Nicotiana rustica* peut être ainsi un hôte sans symptômes pour le Stolbur (M.T. Cousin et J.P. Moreau, 1965) et Gianotti *et al.*, on observé des mycoplasmes chez des plantes d'apparence saine (*in* Caudwell *et al.*, 1971). « On peut (même) supposer... l'existence de plantes et de vecteurs relais, plus ou moins persistants, réservoirs de germes, permettant aux maladies de passer d'une espèce végétale à une autre, source d'infection éventuelle pour de nouveaux arthropodes, d'espèce ou même de groupe différents » (Gianotti *et al.*, op. cit.). Ceci pourraît expliquer l'origine même de la F.D., avant sa propagation épidémique avec l'introduction et la multiplication de *S. littoralis*. Là encore, la culture systématique des mycoplasmes devrait permettre d'apporter une réponse.

III - LA TRANSMISSION DU « BOIS NOIR »

Sauf données récentes non encore publiées, on en est encore réduit aux hypothèses en ce qui concerne le ou les vecteurs de cette maladie.

Les faits actuellement connus sont:

1) *Scaphoïdeus littoralis* ne la transmet pas, et d'ailleurs n'a encore jamais été observé dans les régions où sévit la maladie: Bourgogne, Jura, Suisse romande, Moselle (pour autant que les formes de Suisse et de Moselle soient bien le « Bois noir » et non la F.D. proprement dite).

2) La maladie reste longtemps confinée dans un vignoble donné, à un certain nombre de ceps, où elle se répète année après année. Certaines années toutefois, en certains endroits (Caudwell, Gartel) elle se propage sous forme épidémique. Mais ces épidémies ne semblent guère se prolonger ni avoir le caractère « explosif » de celles de la Flavescence Dorée. Elles suggèrent cependant l'existence d'un vecteur aérien.

La situation est un peu analogue à celle du Stolbur en France : le vecteur reconnu, en Europe Centrale comme propagateur épidémique, *Hyalestes obsoletus* Sign. existe, mais apparaît trop tard et en trop petit nombre pour pouvoir être impliqué dans la propagation de la maladie. En revanche, *H. obsoletus* a été observé, en populations importantes sur les racines de diverses Labiacées et sur celles du Lavandin (*Lavandula officinalis* Chaix × *Lavandula latifolia* Vill.) et serait peut-être responsable de la propagation du « Dépérissement » du Lavandin.

Le « Bois noir » devant être considéré désormais comme une maladie différente de la F.D., il faut considérer aussi, *a priori* que les données acquises sur cette dernière (carastéristiques de la transmission, période d'acquisition, durée de latence, localisation de l'agent infectieux dans les tissus, etc...) ne peuvent être directement transposées.

La stabilité de la maladie sur certains ceps a pû suggérer qu'elle se maintenait par réinoculations successives par des vecteurs sédentaires. Certains Acariens de la vigne (*Eriophyes*, *Puyllocoptes*, *Epitrimerus*) présentent ces caractéristiques et *Phytoptus ribis* Nal est vecteur de la « Réversion » du Cassis (Tresh, 1970).

Néanmoins cette hypothèse paraît peu plausible. De plus, elle repose, en partie, sur l'assertion selon laquelle la réinoculation est obligatoire pour que la maladie se maintienne. Or en ce qui concerne du moins la F.D., nous ne sommes pas aussi absolus que Caudwell sur ce point et pensons avoir démontré (Schvester, 1969; Schvester, Carle, Moutous, 1969) que la maladie pouvait chez un certain nombre de ceps, se maintenir plusieurs années sans qu'une réinoculation soit nécessaire.

Les manifestations épidémiques, généralement localisées dans le temps et dans l'espace suggèrent l'intervention de multiplications momentanées et (ou) locales d'une espèce, se montrant, à cette occasion un vecteur efficient.

Il faut remarquer, par analogie, non seulement avec la F.D., mais aussi avec plusieurs maladies de plantes pérennes, que lorsque l'épidémie est constatée, et compte tenu de la durée d'incubation de la maladie chez la plante, la pullulation occasionnelle de cette espèce responsable hypothétique, s'est probablement produite l'année précédant la constatation, et qu'ainsi sa détection peut échapper à l'observateur.

Dans le Jura précisément, il y a plusieurs années, nous avons personnellement observé, conjointement avec PIGNAL, qui en a rendu compte (1954), des pullulations inhabituelles sur arbres fruitiers et sur vigne de *Cercopides* (*Cercopis* sp.). Les Insectes de ce groupe apparaissent généralement comme se nourrissant dans le xylème, et d'autre part, les blessures profondes que leurs stylets provoquent dans les tissus, nuisent peut-être à une bonne efficience comme vecteurs. (HILDEBRAUD, 1963).

Nous ne rapportons le fait qu'à titre d'exemple d'une pullulation occasionnelle, de même que BONFILS et LECLANT (1972) rapellent les dommages observés en 1880 par BLANCHARD avec *Hysteropterum bilobum* FIEB, et par AUDOUIN en 1890, avec *Penthimia nigra* Goeze. De tels faits ne sont donc probablement pas absolument exceptionnels. D'ailleurs, les quelques essais de transmission de la F.D. avec *Ptyelus spumarius* L. et *Cercopis* sp. ont été négatifs, bien que ces espèces survivent bien et puissent même se développer sur la vigne.

L'efficacité d'une transmission dans la nature implique, en effet, d'une façon générale que l'espèce vectrice ait un comportement de nutrition qui la porte naturellement à s'alimenter sur l'espèce végétale considérée (même si d'ailleurs, elle est polyphage) et non pas seulement qu'elle puisse y survivre un temps plus ou moins long, dans des conditions artificielles. C'est ainsi, par exemple, que le « Rubus stunt » ne peut être naturellement transmis au fraisier par *Macropsis fuscula,* qui ne vit pas normalement sur cette espèce. De même, c'est parmi les Insectes-hôtes normaux du Lavandin que MOREAU et LECLANT (1972) ont détecté une espèce effectivement vectrice du « Dépérissement » : *Cechenotettix martini* Leth. De plus. le temps d'adaptation le plus souvent nécessaire à un Insecte (Cf. « Host selection principle ») lors du passage d'un hôte à un hôte très différent peut être de nature à resteindre encore les possibilités de transmission.

C'est donc, parmi des espèces à comportement normalement ampélophage, fut-ce épisodiquement, qu'il convient, à notre avis, de rechercher en premier lieu les vecteurs possibles. Les espèces trouvées dans les vignobles peuvent être nombreuses (Bonfils et Schvester, 1960; Batiashvili et Dekanoidze, 1967) mais le comportement trophique réel de toutes les espèces est loin d'être entièrement connu.

Peut-être faudrait-il rechercher l'Insecte vecteur dans d'autres groupes que les Cicadelles? Certains Psyllides par exemple: *Psylla pyricola* a été reconnu vecteur du « Pear decline » (Jensen *et al.*, 1964) et *Trioza nigricornis* transmettait une prolifération de la carotte (Marchoux *et al.*, 1972). D'autre part, Silvere et Tits (1969) et Silvere (1970) (cité par Maramorosc *et al.*, 1970) ont retrouvé des corpuscules « interprétés comme des mycoplasmes détériorés » non seulement chez *Phytoptes ribis*, mais aussi chez un thysanoptère souvent associé aux Cassis atteints de « Reversion »: *Thrips fuscipennis*.

IV - LA DISSEMINATION DE VECTEURS ET DES MALADIES

La coïncidence des aires de dispersion des vecteurs et des maladies qu'ils transmettent, est souvent loin d'être parfaite. Ainsi, l'aire de *S. littoralis* déborde très largement celle où la F.D. « *sensu stricto* » s'est manifestée épidémiquement. Inversement, des jaunisses analogues à la F.D. se manifestent en des points encore très éloignés de l'aire de l'Insecte. De même, par exemple, *Scleroracus vaccinii*, vecteur du « faux bouton » de l'airelle (« Cranberry false blossom ») n'est-il distribué que dans les aires de culture les plus anciennes (Wisconsin en particulier) et non dans les Etats de Washington et d'Oregon, où cependant la maladie existe, par introduction de plants infectés. (Stevens, 1931, *in* Stretch, 1970).

De tels fait mettent en évidence les problèmes de dissémination. La limitation de l'extension épidémique revient à éviter, si possible, la rencontre des vecteurs avec celle des agents infectieux. Au mieux, en évitant la dissémination de l'un et de l'autre, ou, à la riguer en évitant la dissémination de l'un seulement. Ceci paraît d'autant plus simple (au moins relativement) que la spécificité

vecteur-agent infectieux ou (et) la spécificité vecteur-hôte végétal est plus étroite.

L'exemple malheureux de l'extension récente de la F.D. sous forme épidémique en Corse, est probablement le résultat d'une introduction simultanée ou presque.

Les Insectes qui, comme *M. fuscula* ou *S. littoralis* hivernent à l'état d'oeuf peuvent être véhiculés, par mégarde, à de grandes distances, particulièrement facilement: ainsi *S. littoralis* pond naturellement ses oeufs de préférence sur les bois de deux ans et plus, mais peut aussi les déposer même sur des bois de l'année, dès lors qu'ils présentent surtout quelque fente ou quelque anfractuosité. C'est très probablement par ces voies qu'il fut introduit, puis dispersé en Europe. La diapause de l'oeuf confère à l'espèce une plasticité biologique qui lui permet de supporter, même des hivers très rudes et aussi de s'adapter à des climats de type méditerranéens. *S. littoralis* n'a qu'une génération annuelle, mais les expériences de modification du cycle naturel (Caudwell *et al.*, en particulier) suggèrent même que, surmontant l'obstacle de l'inversion des saisons, il pourrait éclore et s'installer dans l'hémisphère Sud, si des oeufs y étaient apportés. Inversement cependant, ces transports pourraient être aisément évités par un traitement approprié, combiné au besoin avec une quarantaine.

Parallèlement, les transports de bois, de plants, atteints de jaunisses devraient pouvoir être éliminés, moyennant les précautions classiques. Un point cependant, demeure: il est frappant de constater que jamais personne, à notre connaissance, n'ait signalé de symptômes de F.D. sur des variétés de porte-greffes.

En Gascogne, pourtant, les vignes greffées atteintes présentaient souvent des rejets de porte-greffe, toujours apparemment indemnes. Ceci suggère la possibilité que peut-être, certaines variétés ou espèces au moins, puissent héberger l'agent infectieux sans extérioriser de symptômes. Si cela était, il y aurait là une voie particulièrement insidieuse de dispersion de la maladie. L'existence de mycoplasmes, observée (cf. supra.) chez certaines espèces végétales qui demeurent cependant exemptes de symptômes, semble donner du poids à cette hypothèse dont la vérification cependant n'a, à notre connaissance, pas été entreprise.

RIASSUNTO

Insetti vettori delle malattie da virus e da micoplasmi della vite

In questo lavoro viene esaminato il problema dei vettori della « Flavescence dorée » (FD) e del « Bois Noir » (BN), malattie fra loro simili. Vengono riportati dati relativi alla trasmissione della FD per mezzo dello *Scaphoideus littoralis* in campo e in serra. Si riferisce inoltre sui tentativi di trasmissione della FD in campo, da vite a vite, a mezzo di altri vettori quali *Euscelis plebejus* e *Aphrodes bicinctus*.

Per quanto riguarda il BN l'A. ricorda che tutti i tentativi di trasmissione finora effettuati per mezzo dello *S. littoralis* hanno dato esito negativo; quindi discute la possibile esistenza di altri vettori e formula nuove ipotesi in argomento.

L'A. infine accenna ad alcuni problemi epidemiologici connessi con i rapporti FD - *S. littoralis*.

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UKRAINEAN RESEARCH INSTITUTE OF VITICULTURE AND ENOLOGY, ODESSA

THE NEMATODE FAUNA IN THE NORTHERN BLACK SEA ZONE

B.N. MILKUS, P.M. SHTERENBERG, D.S. TSHEREVKOVA

The soil samples were taken during july-december 1972 in the root zone up to 1 meter depth. The nematodes were extracted immediately after sampling by the funnel-flotation method. The observations were conducted on the living objects as well as on those fixed in the 4% formalin

In the vineyards, situated on southern chernozem (Ukrainean Research Institute of Viticulture and Enology, Odessa) nematodes belonging to 16 genera from 13 families had been found. The nematodes found represent all the five ecologic groups according to PARAMONOV's (1964) classification (Table 1).

In the different periods of sampling the group of *pararhizobiontes-longidorides* prevailed. *Xiphinema index* Thorne and Allen lives on the roots of healthy as well as on the roots of vines infected by fanleaf and infectional chlorosis viruses. It lives also on the roots of birch, rose, apple and rarely thuja trees. *Xiphinema index* was also found in the rhizosphere of vines growing on the sandy loam of the left Dnieper bank (Tsurupinsk, Kherson district, Experimental station of afforestation and viticulture on sands). The

Table 1.

Composition of the ecologic groups of nematodes on grapevine

Ecologic group	Southern chernozem		Sandy loam	
	species in soil	species on roots	species in soil	species on roots
Pararhizobionts	9	8	3	3
Eusaprobionts	1	2	1	1
Devysaprobionts	3	3	3	1
Phytohelmints of nonspecific pathogenic effect	4	4	3	3
Phytohelmints of specific pathogenic effect	1	1	—	—
Total species	18	18	10	8

species *Xiphinema vuittenezi* Luc, Lima, Weischer et Flegg, is widely represented. It occurs separately and together with *X. index*. By the population density it often overcomes the later. Separate specimens occur at the depth up to 1 m. The species *Xiphinema diversicaudatum* (Micoletzky) Thorne and *Xiphinema americanum* Cobb occurred in small quantities. In the samples taken in december many *Xiphinema* sp. larvae could be found.

In the rhizosphere of vines *Longidorus elongatus* (de Man) Thorne et Swanger and unidentified species of this genus were also found, but their number prevailed in samples taken under trees and ornamental shrubs (birch, chesnut, rose, jasmine).

The southern gallforming nematode *Meloidigine incognita* (Kofoid et White) Chitwood has also been found on the roots of vines. From Eusaprobionts the represantatives of *Rhabditidae* fam. occurred. From devisaprobionts - *Cephalobus* Bastian, *Eucephalobus* Steiner and *Acrobeles* genera were found.

In the soil and on the roots predatory nematodes *Mononchus* sp. had been found (Table 2).

TABLE 2.

The species composition of nematod fauna on southern chernozem and sandy loam

	Southern chernozem		Sandy loam	
	in soil	on roots	in soil	on roots
Fam. Chromadoridae				
Prochromadorella viridis	—	+	+	—
Fam. Alaimidae				
Alaimus primitivus	+	+	+	+
Fam. Mononchidae				
Mononchus sp.	+	—	—	—
Fam. Longidoridae				
Xiphinema diversicaudatum	+	+	—	—
Xiphinema americanum	+	+	—	—
Xiphinema index	+	+	+	+
Xiphinema vuittenezi	+	+	+	+
Longidorus elongatus	+	—	—	—
Longidorus sp.	+	+	—	—
Fam. Dorylaimidae				
Dorylaimus sp.	+	+	—	—
Discolaimus sp.	+	+	+	+
Fam. Criconematidae				
Criconema rusticum	+	+	+	+
Fam. Heteroderidae				
Meloidogyne incognita	+	+	—	—
Fam. Tylenchorhynchinae				
Tylenchorynchus sp.	+	+	—	—
Fam. Aphelenchidae				
Aphelencus avenae	+	+	—	—
Fam. Aphelenchoididae				
Aphelenchoides sp.	+	+	+	+
Fam. Diplogasteridae				
Diplogaster sp.	—	+	—	—
Fam. Rhabditidae				
Rhabditis sp.	+	+	+	+
Fam. Cephalobidae				
Cephalobus sp.	+	+	+	+
Eucephalobus elongatus	+	—	—	—
Fam. Acrobelinae				
Acrobeles ciliatus	+	+	—	—
Fam. Trichodoridae				
Trichodorus sp.	—	—	+	+
Fam. Cyantholaimidae				
Choanolaimus osamophilus	—	—	+	—

Conventional signs: + presence of the species,
— absence of the species.

RIASSUNTO

Nematodi presenti in vigneti della zona a nord del Mar Nero

Gli esami sono stati compiuti su campioni di terreno prelevati fino a 1 m di profondità nel periodo luglio-dicembre 1972. Nei vigneti del chernozem meridionale sono stati riscontrati 16 diversi generi appartenenti a 13 famiglie. *Xiphinema index* è risultato presente su radici sia di viti sane che malate, come pure su radici di betulla, rosa, melo e tuja.

Nei suoli sabbiosi della riva sinistra del Dnieper è frequente *X. vuittenezi*, accompagnato o no da *X. index*.

Sono pure presenti *X. diversicaudatum*, *X. americanum* e *Longidorus elongatus*.

LANDES-LEHR- UND FORSCHUNGSANSTALT FÜR WEIN- UND GARTENBAU,
NEUSTADT/WEINSTR.

VORKOMMEN VON *XIPHINEMA*-ARTEN IN WEINBAUGEBIETEN VON PFALZ UND RHEINHESSEN

MARIA RÜDEL

Das pfälzische Weinbaugebiet, von der französischen Grenze bis Bockenheim, westlich von Worms reichend, zeichnet sich durch eine für deutsche Verhältnisse günstige Klimalage aus. Es ist mit einer mittleren Jahrestemperatur von 10,1 bis 10,5 Grad C und einer mittleren Jahresschwankung von nur 18,1 Grad C nicht nur die wärmste Gegend Deutschlands, sondern hat auch eine gleichmässigere Witterung als z.B. Südtirol.

Rheinhessen schliesst sich nördlich an die Pfalz an und erstreckt sich bis in die grosse Rheinschleife zwischen Mainz und Bingen. Die durchschnittliche Jahresmitteltemperatur liegt bei 9,8 Grad C, das Gebiet ist im Sommer recht heiss und trocken.

Seit dem Jahr 1970 haben wir in den genannten Weinbaugebieten von Pfalz und Rheinhessen nematologische Untersuchungen durchgeführt und die folgenden *Xiphinema*-Arten nachweisen können:

— *Xiphinema vuittenezi*
— *X. index*
— *X. mediterraneum*
— *X. diversicaudatum*

Die Reihenfolge dieser Aufzählung entspricht der Häufigkeit des Vorkommens der einzelnen Arten. Ihre Fundorte sind in Abb. 1 dargestellt (vgl. auch Tabelle 1).

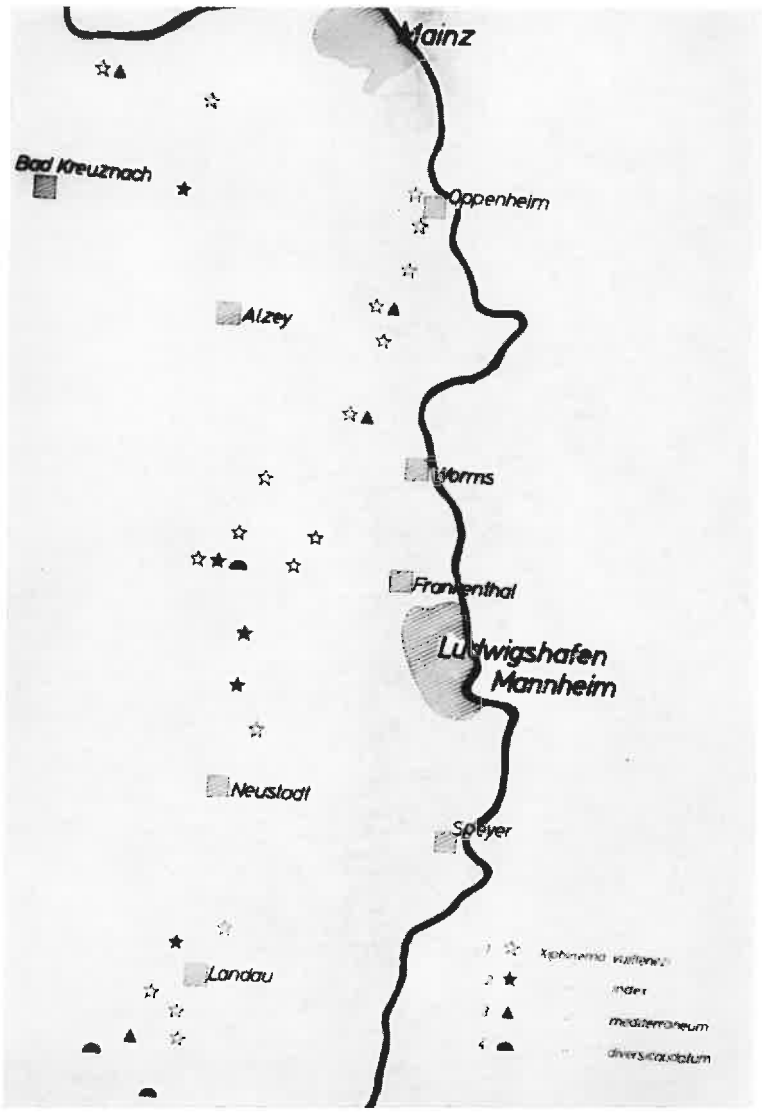


Abb. 1. - Verbreitung von *Xiphinema*-Arten in Pfalz und Rheinhessen

TAB. 1.

Vorkommen von *Xiphinema*-Arten in Pfalz und Rheinhessen

Arten	Anzahl untersuchter Flächen		
	weinbaulich genutzt		landwirtschaftl. genutzt
	gesamt	+ Virosen	
	166	92	28
Xiphinema vuittenezi	74 *)	40 *)	16
X. index	40 *)	38 *)	—
X. mediterraneum	8	3	2
X. diversicaudatum	3	3	1

*) 2 × Mischpopulationen

XIPHINEMA VUITTENEZI LUC, LIMA, WEISCHER ET FLEGG 1964

Diese Art ist bei uns offensichtlich am weitesten verbreitet (Abb. 1, Tab. 1). Ihr Wirtspflanzenkreis ist relativ gross, womit auch ihre Häufigkeit zu erklären ist.

Wir beobachteten *X. vuittenezi* im Freiland an folgenden Pflanzen:

— Kartoffel (bis zu 220 Adulte / 250 ccm Boden)
— Zuckerrüben (» » 129 » / 250 ccm »)
— Grasgemisch (Wegrain) (» » 110 » / 250 ccm »)
— Reben (» » 100 » / 250 ccm »)
— Getreide (» » 71 » / 250 ccm »)
— Mais (» » 37 » / 250 ccm »)
— Aprikosen (» » 13 » / 250 ccm »)

Im Topfversuch hielten sich *vuittenezi*-Populationen auch an Ölrettich, Liho-Raps, Sonnenblume und Luzerne.

Die Art hat nach unseren bisherigen Beobachtungen die grösste Populationsdichte an Kartoffel (mit 220 Adulten/250 ccm Boden), und eine geringere an Reben mit höchstens 100/250 ccm Boden erreicht. Gemessen an den Populationshöhen der übrigen *Xiphinema*-Arten ist *X. vuittenezi* jedoch auch bei der Rebe noch relativ stark vertreten. Über Winter tritt keine sehr wesentliche Reduktion der Anzahl ein.

X. vuittenezi bevorzugt Lösslehme und erreicht hier die grössten Populationsdichten in Verbindung mit einer geeigneten Wirtspflanze.

Ein Zusammenhang zwischen *X. vuittenezi* und der Übertragung von Rebviren ist noch immer nicht eindeutig geklärt, obwohl er schon gelegentlich vermutet worden ist. Nach unseren Beobachtungen und Untersuchungen kommt *X. vuittenezi* häufig in Beständen mit sich ausbreitender Reisigkrankheit vor, ohne dass *X. index* oder ein anderer möglicher Vektor nachgewiesen werden konnte: (Tab. 1). Bei zahlreichen Übertragungsversuchen im Gewächshaus gelang nur in einem Fall der Nachweis eines Virus in Wurzeln ursprünglich gesunder *Rupestris* du Lot, die mit *X. vuittenezi* aus einem Reisigherd besetzt worden war. Das Virus verursachte aber auf der Test-pflanze *Ch quinoa* ein atypisches Symptombild, und es sind noch weitere Untersuchungen notwendig.

XIPHINEMA INDEX THORNE ET ALLEN 1950

Diese Art wird vorwiegend in Qualitätsweinbaugebieten beobachtet, d.h. auf Flächen, die klimatisch begünstigt sind und normalerweise ohne Brache weinbaulich genutzt werden. Noch nie fanden wir *X. index* in vormals landwirtschaftlich genutzten Flächen oder in Anbaugebieten mit Mischkulturen. Damit ist der Wirtskreis dieser Art bei uns offensichtlich auf die Rebe beschränkt (Tab. 1, Abb. 1).

Im Freiland baut *X. index* nur relativ schwache Populationen auf, Werte von 50 Adulten je 250 ccm Boden sind bereits selten. In Topfversuchen im Gewächshaus kann sie dagegen höhere Populationsrichten erreichen.

X. index ist als Vektor des Komplexes Reisigkrankheit bekannt, und auch in unseren Gebieten mit Ausnahme von nur zwei Fällen immer in Beständen gefunden worden, in denen sich Reisigkrankheit und Infektiöse Panaschüre ausbreiteten. Die schnelle

Übertragung von Virus lässt sich im Freiland an der starken Zunahme viruskranker Stöcke innerhalb weniger Jahre feststellen. Im Gewächshaus wurden Übertragungen vom Juli, dem Zeitpunkt der Inokulation, bis zu Beginn der folgenden Vegetationsperiode durch die Ausprägung von Symptomen an *Rupestris* du Lot und FS 4 manifest (Abb. 2).

Abb. 2. - *Rupestris* du Lot, ein Jahr nach Inokulation durch *Xiphinema index* von Spätburgunder mit Reisigkrankheit und Infektiöser Panaschüre

XIPHINEMA MEDITERRANEUM LIMA 1965

X. mediterraneum wurde bisher nur selten (Tab. 1) und in weit voneinander entfernt liegenden Gebieten beobachtet (Abb. 1). Bis auf zwei Vorkommen auf landwirtschaftlichen Flächen (Kartoffeln, Mais) und einem unter Ungräsern eines Wegrandes wurden die übrigen an Reben registriert. Die Populationsdichte schwankte zwischen 203 Adulten/250 ccm Boden unter Ungräsern und 70/250 ccm an Reben bis zu 3/250 ccm, ebenfalls an Reben, sie ist also, gemessen and der in südlichen Klimaten relativ gering.

Ein Zusammenhang zwischen *X. mediterraneum* und Rebvirosen konnte bisher nicht eindeutig ermittelt werden, obwohl die Art dreimal in reisigkranken Beständen gefunden wurde. Übertragungsversuche im Gewächshaus haben bisher keine eindeutigen Ergebnisse gebracht.

XIPHINEMA DIVERSICAUDATUM (MICOL. 1927) THORNE 1939

Bisher nur in drei Weinbergen und in einer früheren Obstanlage innerhalb der Pfalz wurde *X. diversicaudatum* beobachtet, und dies jeweils nur in sehr geringen Anzahlen. Obgleich die Art an zahlreichen Pflanzen existieren kann, scheint doch die Rebe kein besonders günstiger Wirt zu sein.

Inwieweit die Art als Vektor für Rebviren fungieren kann, lässt sich auf Grund eigener Untersuchungen bisher nicht festlegen. Wir fanden sie allerdings jeweils in sich vergrössernden Virusherden. In zwei Fällen verursachten die Viren sowohl auf den Reben als auch auf den Testpflanzen abweichende Erkrankungen, in einem dritten Fall wurde Ring- und Linienmuster an den Reben beobachtet, hier allerdings lag eine Mischpopulation mit *X. index* vor.

SUMMARY

In the vine-growing areas of Pfalz and Rheinhessen the following species of *Xiphinema* have been found: *X. ruittenezi*, *X. index*, *X. mediterraneum*, *X. diversicaudatum*.

X. ruittenezi was the most aboundant species, it occurs in very high numbers, and apparently with a wide host-range. Hits ability of transmitting viruses has not been determined exactly as yet.

X. index has been detected mainly within zones of high wine quality, it occurs mostly in low numbers, and its only host in Germany seems to be the grape. The transmission of grape-viruses by *X. index* has been established also in our regions, in the field and in the glass-house.

X. mediterraneum and *X. diversicaudatum* have been found rarely and always in low numbers in association with grapes and the letter widely scattered. Transmission of virus by these species could not be proved exactly.

RIASSUNTO

Specie di Xiphinema *presenti nei vigneti del Palatinato e della Renania*

Nella zona viticola del Palatinato e della Renania sono state riscontrate le seguenti specie di *Xiphinema*: *X. vuittenezi*, *X. index*, *X. mediterraneum*, *X. diversicaudatum*. La specie più diffusa e più frequente è risultata essere *X. vuittenezi*, che presenta un ciclo di piante ospiti piuttosto vasto. La sua capacità di trasmettere virus non è ancora stata esattamente determinata.

X. index è stato riscontrato prevalentemente nelle zone di vini ad alta qualità e in quantità ridotte; sembra che in Germania viva soltanto su vite. La trasmissione di virus della vite attraverso questa specie è stata accertata anche nelle nostre regioni sia in campo che in serra.

X. mediterraneum e *X. diversicaudatum* sono risultati essere poco diffusi e poco numerosi. La trasmissione di virus attraverso queste specie non si è potuta dimostrare

INSTITUTE FOR PLANT PROTECTION - BEOGRAD, YUGOSLAVIA

SOME OBSERVATIONS ON NATURAL SPREAD OF GRAPEVINE LEAFROLL DISEASE IN YUGOSLAVIA

B. DIMITRIJEVIC

INTRODUCTION

There are no many dates about the mode of natural spread of Grapevine leafroll disease. Although the infectious nature of the disease was established long time ago (Scheu, 1936), its cause is not determined until now and little is known also about its vectors. Unpublished results obtained by Dias (1962) in Portugal, as well by Chiarappa (1963) in California, about transmission of Grapevine leafroll by mealy bugs (*Pseudococcidae*), at the moment could be only indications for further investigations (Hewitt, 1968).

The majority of the scientists consider that causal organism of Grapevine leafroll can be transmitted by infected plant materials only, especially through American rootstocks, which can be symptomless carriers of virus (Goheen *et al.*, 1958; Bovey, 1970; Boubals, 1970; Goheen, 1970; Hewitt, 1971).

Investigations carried out during last three years, after observing Grapevine leafroll in Yugoslavia (DIMITRIJEVIĆ, 1970), indicated that natural spread of this disease may be also of certain epidemiological importance, which has to be considered.

Our results of these investigations are presented in this paper.

MATERIAL AND METHOD

Observations were carried out in a vineyard near Beograd, over fifteen years old, established in a system of espalier, with 3 m distance between the rows, and about 1,5 m between the plants. It was surveyed a plot with seven rows of Gamay. As it is known, the infected plants of this cultivar of grapevine show very pronounced symptoms of leafroll disease: premature blue-red coloration and inward rolling of leaves. In each row of the plot were planted 105 vines, but after planting about 15% of the plants were destroyed and so remained 626 vines totally. Observations were carried out during 1970, 1971 and 1972, at least twice a year, in August-September, when the symptoms of the disease were the most pronounced.

During the investigations a special attention was paid to all factors which could be, eventually, the cause of similar symptoms (leafhoppers, mechanical damages) and could provoke erroneous conclusions. There were also excluded plants of other cultivars, like those with natural red color of leaves (« teinturier »). In the cases of doubtful symptoms, it was counted as diseased only the plant which clearly pronounced leafroll symptoms in the next year.

RESULTS AND DISCUSSION

The number of leafroll diseased plants estimated on the examined plot in mentioned three successive years, are presented in tab. 1.

In the first year it was found 14 diseased vines, and in second one 25, which means that its number increased about 80% during

TAB. 1.

Number of plants infected by Grapevine leafroll disease in a vineyard near Beograd, with 626 vines of Gamay during three successive years.

[+ = clear disease symptoms (+) = doubtful disease symptoms]

Row	No. of vines	Occurrence of Leafroll			Notes
		1970	1971	1972	
I	57	—	(+)	+	
	78	+	(+)	+	
II	58	—	+	(+)	dead
	63	—	(+)	+	
	74	+	(+)	+	
	75	+	+	+	
	79	—	+	+	
	80	+	+	+	
	87	—	—	+	
	95	—	(+)	+	
III	37	+	+	+	
	63	—	(+)	+	
	70	—	—	+	
IV	5	—	—	+	
	22	—	(+)	+	
	32	(+)	(+)	+	
	35	—	—	+	
	52	(+)	+	+	
	62	+	+	+	
	73	(+)	(+)	+	
	78	(+)	(+)	+	
	82	+	+	+	
V	22	—	—	+	
	43	—	(+)	+	
VI	20	+	(+)	+	
	41	—	(+)	+	
	58	—	+	+	
	76	—	—	+	
	89	+	+	+	
VII	52	—	+	+	
	59	+	+	+	
Total		14 (2,2%)	25 (4%)	31 (5%)	

one year. In the third year it was found 31 vines with clear symptoms of the disease. As can be seen, the number of the diseased plants was increased about 25% in this year.

The evidence obtained in this investigations indicated that the number of leafroll diseased vines were increased relatively fast annually, so that during two years it was more than doubled. Further investigations will show whether the spread of the disease will have the same tendency.

Although the incubation periode of Grapevine leafrol disease can be rather long, it is considered that its duration does not last more than three years (GOHEEN *et al.*, 1958; STELLMACH, 1968). Thus, it is impossible that all the diseased vines in the examined vineyard, which is over 15 years old, were infected before planting.

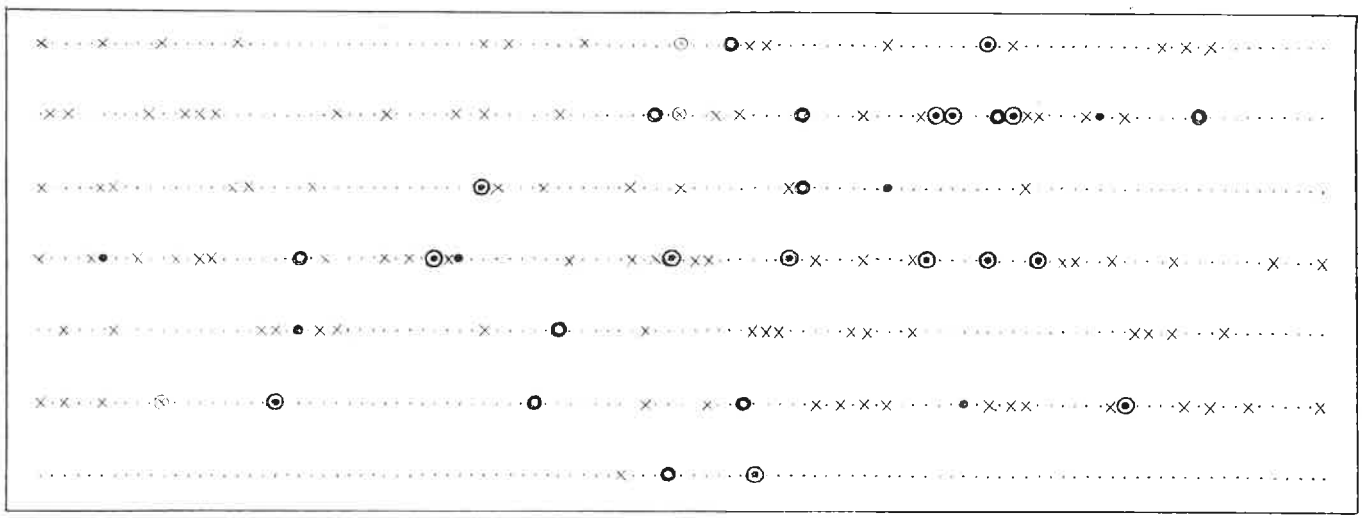
It could be supposed that we had a mild strain of virus, or very pronounced tolerance of some grapevines to this disease, so they were long time symptomless carriers of the virus. In that case it is not clear, what is the cause of the changes in the relationship between the pathogens and plants, and why suddenly the pronounced manifestation of the disease occurs on the great number of plants.

It will be difficult to explain this phenomenon until to the modes of natural spread of Grapevine leafroll disease are discovered. If under our conditions it is realy the matter of new infections of previously healthy plants, as it seems, it must exist a vector which transmit the cause of the disease. At the moment it could not be said anything about these vectors, but on the base of the pattern of disease spread (Fig. 1), it seems more probably that the cause of the disease is air-borne than soil-borne.

CONCLUSION

Observations of the appearance of the Grapevine leafroll disease during three sucessive years, in a vineyard over 15 years old, near Beograd, showed that the number of the diseased vines from 1970 to 1972 was more than duplicated. It means that under some conditions natural spread of Grapevine leafroll disease may have rather high epidemiological importance.

Fig. 1 – Distribution of diseased vines in a plot of Gamay in tree successive years in a vineyard near Beograd (Yugoslavia)



⊙ vines with leafroll disease symptoms in 1970, 1971 and 1972
o vines with leafroll disease symptoms only in 1971 and 1972
• vines with leafroll disease symptoms only in 1972
· vines without symptoms
× missing vines ⊗ other varieties of vines

RIASSUNTO

Alcune osservazioni sulla propagazione naturale dell'accartocciamento fogliare della vite in Yugoslavia

Molto poco si sa sul modo di propagazione naturale dell'accartocciamento fogliare della vite. Solitamente si considera che il virus che causa questa malattia venga trasmesso attraverso il materiale viticolo infetto (legno e barbatelle). Le osservazioni effettuate nel corso di tre anni su un vigneto di 15 anni, situato nei pressi di Belgrado, sembrano indicare che non è da scartare la possibilità di una propagazione naturale per mezzo di qualche vettore. In una parcella con 626 viti di Gamay il numero delle piante malate è cresciuto, in modo permanente, ogni anno: 14 ceppi infetti nel 1970, 25 nel 1971 e 31 nel 1972. La distribuzione delle viti malate nei tre anni sembrerebbe indicare che la malattia è diffusa per via aerea.

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SESSIONE III

METODI DI STUDIO

Methods in research

(*chairman*: R. Bercks)

STATION DE PATHOLOGIE VEGETALE - CENTRE DE RECHERCHES I.N.R.A.
68021 COLMAR (FRANCE)

METHODS OF STUDYING VIRUS DISEASES OF GRAPEVINES

A. VUITTENEZ

I. - INTRODUCTION

The different techniques which can be used for the study of viruses associated with degeneration producing diseases of the Grapevine have been considerably developed in the last few years.

They include first transmission of various types of symptoms to the vine through grafting or animal vectors, and also transmission by sap inoculation to herbaceous hosts, purification with physical or chemical methods and serological study of some specific Grape viruses, especially those of the « ringspot »-type soil borne and transmitted by nematodes (NEPO viruses). The results thus obtained have led to the elaboration of the present diagnosis techniques, such as indexing on Grape indicator varieties and laboratory tests restricted to isolable viruses.

For many transmissible diseases of the Grapevine or other plants with virus-like symptoms, the usual virological methods have failed to reveal the causal agent, but new techniques are now developed such as ultrastructural observation of infected tissues with the electron microscope. In some cases they have already allowed the discovery of particles, probably the infectious agents themselves, some of which were proved to be, in fact, different from viruses.

Thus, the research on Grapevines is concerned with a large variety of techniques to be applied in the fields as well as in the greenhouse or the laboratory.

We intend to sum up here some of the most recent information, restricted to virus diseases or to those we reasonably suppose to be so.

II. - TECHNIQUES BASED ON OBSERVATION OF VISUAL SYMPTOMS IN GRAPEVINES

A. *Types of symptoms*

New types of symptoms are now considered to be related to virus infection of Grapevines. They concern not only characteristic changes in organs like leaves, berries, shoots, trunk, bud-union and roots, but also more general factors such as vigour, grafting success and longevity of some varieties when grafted on certain rootstocks.

New terms are necessary for a proper description of fine variations affecting the discoloration of leaves, showing different degrees and patterns of mosaic, reddening or necrosis. The same applies to the description of symptoms on the wood surface. The difficulty lies in describing the successive symptoms composing the syndrom of individual diseases and finding a suggestive name for them. This is all the more critical as the symptoms can vary according to the virus strains, to the variety of inoculated Grapevines as well as to climatic conditions or time of observation after inoculation.

B. *Inoculation by grafting of Grape varieties*

Inoculation by grafting is the basic and still very fruitful technique to conclude to the viral nature of symptoms observed on Grapevines and to discover new unexpected viruses.

Grafting techniques especially by « chip-budding » on the side or preferably at the base of cuttings of dormant wood are now perfected. The difficulty is rather in the interpretation of the results of the transmission, owing to the existence of undesirable, mostly unsuspected viruses, either in the material which is to be inoculated or in the inoculum itself. The only way to reduce mistakes in interpretation is to repeat each experiment several times with different clones of Grapevines used as donors or receptors. So we can make

sure that the symptoms observed on inoculated grapevines are actually produced by the virus under study and not by another virus already present in the wood of the inoculated grapes or in the inoculum. The simultaneous presence of other different viruses in the inoculum which are transmitted along with the virus under study, can lead to a false relation of the symptoms induced in the range of inoculated plants; it can be said that such confusions have certainly occurred in the past.

The main interest of systematic grafting experiments lies in the revealing of viruses latent in many varieties of Grapevines but able to cause severe disease when transmitted to certain other varieties used as indicators. So the « Marbrure », latent in *V. vinifera* and many rootstocks varieties, induces mosaic of the finest veins, a distorsion of the surface of the leaves and sometimes a severe stunting in *V. rupestris* or *rupestris-Berlandieri* 99 R. Another type of mosaic affecting *V. vinifera* as well as the rootstocks is readily detected by indexing on *V. riparia*. A third newly discovered affection, latent in many grapevines, produces severe black streaks on the tips of the shoots and black necrosis of the veins of leaves in the indicator variety *rupestris-Berlandieri* 110 R.

The reaction of the indicators to apparently similar viruses is not the same in all environments: for instance the varieties Baco 22 A and LN 33 considered in the USA to be the best indicators of leafroll, are of poor value in Europe as compared to some *Vinifera*. For the « Legno riccio » we lack of sufficient information about the susceptibility of the different rootstock varieties; therefore no method could be evolved up to now for a practical indexing of the disease.

However indexing is already used on a large scale, in several countries, as a routine check against some virus diseases during the sanitary selection of the Grapevine. Results of indexing show that the percentage of clones recognized as infected by « Marbrure » or Leafroll is rather consistent, thus confirming the usefulness of the technique.

C. *Thermotherapy*

The growing of vines at sublethal temperatures during a prolonged period shows that the individual viruses vary greatly in their reaction to heat, providing a possible method for their sepa-

ration. Other more recently discovered affections should also be studied in this respect.

Technically speaking, this method of treatment applied both to small plants cultivated in vitro or to potted plants, has yielded good results for fanleaf and even for leafroll. A big problem is to recuperate a good number of young plants obtained by rooting the tips of treated plants. The in vitro cultivation of these tips has been recently reinvestigated with improved results.

D. *Study of the economical importance of virus diseases on Grapevines*

Inoculation by grafting or thermotherapy, applied to genetically well defined clones, allows comparative field experiments about the effect of known virus diseases as regards growth and production of the grapevines. The effect of fanleaf and leafroll has thus proved to be very important on the most susceptible varieties. There is less quantitative information concerning the other diseases; but it has been demonstrated that diseases such as « Marbrure » considered as latent, have in fact a significant reducing effect on vigour of young grapes in the nursery.

For some viruses the study should be conducted not only on self-rooted grapevines, but also on grapevines grafted on a range of different rootstocks. Virus-related grafting incompatibilities — already familiar in *Citrus, Prunus* and *Malus* — can also occur with some grapevine varieties when grafted on particular rootstocks, for instance with « Legno riccio ». Some cases of leaf reddening followed by stunting and death of scion, have been recently reported consequently to grafting between some clones of *vinifera* varieties and clones of 5 BB rootstocks. The hypothesis of a viral infection connected with such an incompatibility may be formulated and deserves further investigation.

Beside the revealing of obnoxious effects of viruses, another line of research deals with the possible beneficial action of « weaker » virus strains artificially inoculated, which might protect vines against a more severe damage. This would prove particularly useful with « Court-noué »-type viruses when new vines are to be planted in infested surroundings impossible to clean through cultural or chemical methods.

III. - TECHNIQUES BASED ON THE DETECTION OF THE VIRUSES THEMSELVES

A. *Experimental transmission of viruses to herbaceous test plants*

Mechanical transmission of viruses from grapevines, realized for the first time for fanleaf, has consecrated the use of Chenopodiums owing to their high susceptibility to infection. Other plants experimented on later have given better results for other viruses (e.g. *N. Clevelandi* for TMV). Nevertheless, the range of test plants now used shows considerable drawbacks: lack of differential symptoms specific to each virus species, few or no hosts producing local lesions, sometimes total absence of « filter-plants » allowing an efficient separation of different viruses, when they are associated in the same vine, for instance strawberry latent virus and arabis mosaic virus, or fanleaf virus and arabis mosaic virus.

Rather than to search for problematic new test plants, it might be worthwhile to study already known hosts in heat- and light-conditioned rooms. We might obtain stronger and faster responses than in greenhouses and perhaps new types of reactions, which would boost the interest of using test plants for diagnosis purposes. Essentially the possibility of inoculating isolated organs would save time and space during routine sanitary checks, compared with the present use of potted plants. Although isolated *Chenopodium* leaves are not too easy to keep alive on nutrient solutions, preliminary tests conducted in our laboratory have shown that « fanleaf » and « arabis mosaic » viruses are able to develop sufficiently to be detected serologically after a period of 8-10 days.

The retransmission to the Vine of identified viruses isolated after passing on herbaceous hosts has been successful in some cases by means of hetero-specific grafting or mechanical inoculation of young seedlings or of etiolated grapevines; but the larger scale applying of such techniques for a systematic inoculation of the different types of viruses to many varieties of vines — a work already in progress in some laboratories — seems an arduous task. The use of nematodes as vectors might be considered, but the critical point is the availability of an herbaceous plant at the same time host for the virus and accepted by the nematodes.

B. *Purification of viruses*

Methods of obtaining partially purified concentrated extracts of viruses — in particular of the NEPO group — are relatively simple from herbaceous hosts, but much less from grapevine tissues.

Complementary methods of purification vary according to the purposes. For serological use, especially for injection to animals in order to produce antiserums, the main problem is to eliminate the antigenically active protein fraction which originates from the host plant. This can be achieved either by electrophoresis or by serological purification; in this case one should preferably treat the extract with an antiserum specific to the virus. The precipitate formed by the virus bound with antibodies is separated through centrifugation or taken directly by cutting when the reaction takes place in agar gel; after thorough washing it can be used directly for injection. In recent works studying multicomponent-viruses, among which we find the NEPO viruses, special purification techniques have been introduced with a view to separating the various types of particles containing different amounts of RNA, and thus sedimenting at various speeds in a gravity field. These techniques are based essentially on ultracentrifugation in density gradient either by rate zonal centrifugation in sucrose or by quasi-equilibrium in solutions of gradient-forming salts such as cesium chloride or sulphate. This separation of components, sedimenting in very close zones, is sometimes difficult and leads to heavy losses of virus. It is effective for the rather concentrated viruses giving a sufficient yield after purification; but this is unfortunately not the case for many NEPO viruses such as fanleaf. Other techniques are therefore preferred nowadays, separating not the particle types themselves but the various types of RNA. For this, extracts of chemically degraded viruses are submitted to electrophoresis in polyacrylamide gels.

C. *Serology*

As for theoretical aspects, recent researches have mostly concerned the improving of sensitivity of test methods. The purpose is to detect always smaller amounts of antigenes, making easier the tracing of low concentrated viruses in plants and also small amounts of antibodies, in order to study distant relations between viruses or to find small antigenic differences between strains of a

given virus. The latex method has brought about decisive improvement.

The high specificity of serological reaction enables attempts to prepare antiserums able to react to one serotype only within a given virus species. The serums are treated with heterologous antigenes and the remaining strain specific antibodies are concentrated by precipitation. However total specificity cannot always be achieved with all serums. Moreover the use of serology as a quantitative method of determining virus concentration is not completely satisfying yet. It spite of these limitations serology has been applied with some success in our laboratory to the study of interference between different strains of one NEPO-virus or between different virus species of this group.

Apart from this theoretical interest, serological method can be a basis for the elaboration of diagnosis tests for the sanitary control of Grapevines. The two methods of testing — latex and agar gel diffusion after concentration of leaf extracts — have proved to be efficient in routine work. The serological tests can be delayed over some length of time as it is possible to keep in frozen condition the samples of vine leaves taken at the period of maximum virus concentration. On field grown vines, the concentration of fanleaf and arabis mosaic viruses stays high enough over a fairly long period of growth, according to our experiments.

The conclusion of the check by serology or inoculation of test plants over a large body of visually selected vines, originating from different Institutes of Viticulture, reveal an infection rate by NEPO-viruses of just a few percents; this shows the efficiency of visual control for the elimination of « Court-noué » type diseases. This is not the case for latent viruses: the same vine material indexed for other viruses such as « Leafroll » and « Marbrure » reveals a much higher degree of infection (15-25% respectively in our experiments). Unfortunately serology cannot be applied to the detection of these diseases as long as their agents have not been isolated .

D. *Direct observation of viruses and anatomical or ultrastructural changes in infected tissues*

Electron microscope observation of more or less purified preparations from herbaceous hosts experimentally infected by viruses of vines, such as those of the NEPO group, present no difficulty. For

a current operation, like control of concentration and purity of virus in zones from a density gradient, a drop of the preparation is put on a formvar-carbon film coated grid, held horizontally with tweezers; the excess is drained with a slip of filter paper; the sugar is eliminated by pouring a few drops of buffer before staining, or rinsing and staining can be effected simultaneously by pouring buffer containing the negative staining agent such as 1% uranyl acetate (or sodium phosphotungstate, at pH 5 rather than pH 7 for fanleaf virus). The observation of crude saps is possible too, either by the « smear » method or the « dip » method. But both give sometimes rather dirty preparations. Another technique, little used as yet, is te « spreading » which consists in depositing a small quantity of the sample on the surface of a liquid previously dusted with talc powder. The sample spreads in a thin film a part of which is taken away by simply touching it with the grid.

The detecting of round virus particles, especially at low concentration, can be improved by treating the sap with an antiserum specific of the virus. Virus particles then gather in floculates, easier to detect than isolated particles.

The ultramicrotomy techniques, now well perfected, have allowed the direct observation « in situ » of several NEPO-type viruses in infected cells of herbaceous test plants or of the vine itself.

It is thought that for a lot of diseases these direct observation techniques might prove very helpful to try and detect the causal agent, about which nothing is known but its infectivity by grafting.

A preliminary observation by optical microscopy can already be of some use. A particular attention is devoted to the changes observed in the phloem. A good technical model for this can be taken in the study of leafroll. The use of fluorescence microscopy makes easier the detection of necrosis in the phloem in the case of « flavescence dorée » and should be tried for other diseases.

An ultrastructural study of the phloem of Grapevine with electron microscope has been effected in the instance of a disease of « yellows »-type occuring in Germany, but the significance of fibrillar structures observed in some cells is not yet quite clear as regards their possible viral nature. For some diseases much larger sized particles found in infected tissues make interpretation much easier, for instance in the case of rhabdovirales now detected in a lot of plants (e.g. mosaic affected raspberries).

Lastly, real organisms have been found associated with diseases of the Grapevine formerly classified as virus-like affections. This is the case for mycoplasma found in the phloem of plants affected by « flavescence dorée ». Quite recently, even more voluminous particles — assumed to be rikettsia — have been detected in the xylem of vines infected by Pierce's disease. These discoveries direct researches concerning these last types of diseases towards lines different from virological work, involving their own techniques which will be dealt with in another report.

RIASSUNTO

Metodi di studio delle malattie da virus della vite

I principali metodi di studio delle virosi della vite possono essere così riassunti:

1. - *Esame dei sintomi*: effettuato sia direttamente sulle viti malate sia su viti indicatrici inoculate per innesto o per « chip-budding » sia, infine, su viti trattate parzialmente con termoterapia al fine di distinguere i sintomi dovuti a virus resistenti da quelli dovuti a virus sensibili al calore. Interessante può risultare anche lo studio comparativo delle reazioni sintomatiche determinate da ceppi virulenti su piante esenti da virus e su piante precedentemente inoculate con ceppi scarsamente patogeni.

2. - *Rilevamento della presenza e caratterizzazione delle particelle virali*: si possono ottenere attraverso le seguenti vie: trasmissione a piante test erbacee, purificazione, saggi sierologici e diretta osservazione al microscopio elettronico.
In particolare, per quanto riguarda la trasmissione a piante erbacee, oggi appare più interessante lo studio delle risposte sintomatiche su specie adatte ed allevate in condizioni ambientali controllate che non la ricerca di nuove specie test.

Non è infine da trascurare lo studio delle alterazioni istologiche al microscopio ottico, prendendo in particolare considerazione le alterazioni del floema e ricorrendo eventualmente a tecniche recenti, quali la microscopia in fluorescenza.

LANDES-LEHR- UND FORSCHUNGSANSTALT FÜR WEIN- UND GARTENBAU,
NEUSTADT/WEINSTR.

UNTERSUCHUNGEN ÜBER DEN EINFLUSS VIRUSSTABILISIERENDER SUBSTANZEN AUF DIE MECHANISCHE ÜBERTRAGUNG VON REBVIREN

H. BRÜCKBAUER

Die Infektiosität von Blattpressäften aus viruskranken Reben geht unter normalen Bedingungen sehr schnell verloren, und eine Übertragung findet nicht statt.

Nach Dias (1961) ist für das Versagen einer Übertragung eher die Azidität des Rebsaftes verantwortlich als das Vorhandensein eines Hemmstoffes. Demgegenüber vermutet Vuittenez (1959), dass die inaktivierende Wirkung des Rebsaftes auf die Anwesenheit von Gerbstoffen in den Blattpressäften zurückzuführen ist, die durch die in den Blättern vorhandenen Säuren ergänzt wird.

Es ist naheliegend anzunehmen, dass beide Faktoren, also Azidität des Pressaftes und Vorhandensein eines Hemmstoffes, über dessen Nature noch nichts bekannt ist, die Ursachen für die Infektionshemmung sind. Für das Vorliegen eines Hemmstoffes sprechen die Untersuchungen, wonach die Infektiosität des TMV durch Zusatz von Rebpressäften beeinträchtigt wird.

Positive Übertragungen werden erhalten, wenn bei der Herstellung des Inokulums aus Reben eine 2,5%ige Nikotinlösung oder ein 0,07 M Phosphatpuffer pH 8 zugesetzt wird. Der so erzielte Übertragungserfolg gewährleistet jedoch nicht immer die für einen Test erforderliche Sicherheit.

Unsere Untersuchungen hatten zum Ziel, weitere Substanzen auf ihre stabilisierende Wirkung zu prüfen, um eine sichere Diagnose viruskranker Reben zu ermöglichen. Bei der mechanischen Übertragung von Viren aus Obstgehölzen haben sich eine Reihe von Substanzen als infektionsbegünstigende Stoffe erwiesen, die neben anderen Virusstabilisatoren in unsere Untersuchungen einbezogen wurden. Über die erzielten Ergebnisse soll kurz berichtet werden.

MATERIAL UND METHODE

Die für die Übertragungsversuche verwendeten Triebspitzen wurden morgens früh entnommen, gut durchgemischt und bis zur Aufarbeitung bei +4°C aufbewahrt. Jeder Blattprobe von 1 g wurden bei der Herstellung des Pressaftes 3 ccm der Extraktionslösung sowie 5 ccm des jeweiligen Lösungsmittels zugesetzt und vom Inokulum der pH-Wert festgestellt. Die Infektionen erfolgten nach der üblichen Methode auf *C. quinoa* und z.T. auf *C. murale* mit jeweils 5 bis 10 Pflanzen je Serie. Als Kontrolle diente die übliche 2,5%ige wässrige Nikotin-Lösung als Zusatz, mit der bisher die höchste Sicherheit bei der mechanischen Übertragung von Rebviren erzielt wurde.

ERGEBNISSE

1. *Einfluss des pH-Wertes bzw. verschiedener Puffer auf die Infektiosität*

Aus vielen Untersuchungen ist bekannt, dass sich der pH-Wert des Inokulums um pH 8 sehr positiv auf den Übertragungserfolg auswirkt. Bei obstvirologischen Untersuchungen hat der Zusatz von Boratpuffer bzw. Boratpuffer und Stabilisatoren bei manchen Viren zu besseren Übertragungsergebnissen geführt als Phosphatpuffer.

Bei unseren Untersuchungen mit einem stark virulenten Rebisolat (Griesheim, ToSRV) wurden folgende Ergebnisse erhalten (Tab. 1):

Tab. 1.

Einfluss von Borat- und Phosphatpuffer auf die Infektiosität

Stabilisator	Übertragungsrate
Nikotin 2,5% in Wasser	100%
» 2,5% in Phosphatpuffer pH 8	100%
» 2,5% in Boratpuffer pH 9,22	50-60%
Phosphatpuffer pH 8	50-60%
Boratpuffer pH 9,22 (0,01 M)	100%
Polyäthylenglykol 2,5% in Wasser	10-20%
» 2,5% in Phosphatpuffer	100%
» 2,5% in Boratpuffer	50-90%

Die Ergebnisse zeigen, dass mit Boratpuffer ohne Stabilisatoren höhere Übertragungsraten und eine stärkere Symptomausprägung erzielt wurden als mit Phosphatpuffer (Abb. 1, 2). Bei Boratpuffer mit Nikotin oder Polyäthylenglykol war bei niedrigerer Übertragungsrate die Symptomausprägung deutlich stärker als bei Boratpuffer ohne Zusatz.

2. *Einfluss verschiedener Virusstabilisatoren auf die Infektiosität*

Zusätze stabilisierender Substanzen bei der Herstellung der Inokula wirken sich besonders dann günstig aus, wenn die vorher genannten Faktoren, also günstiger pH-Wert und geeigneter Puffer, berücksichtigt werden.

Es wurde eine Reihe verschiedener Stabilisatoren auf ihre Eignung geprüft und folgende Ergebnisse erhalten (Tab. 2):

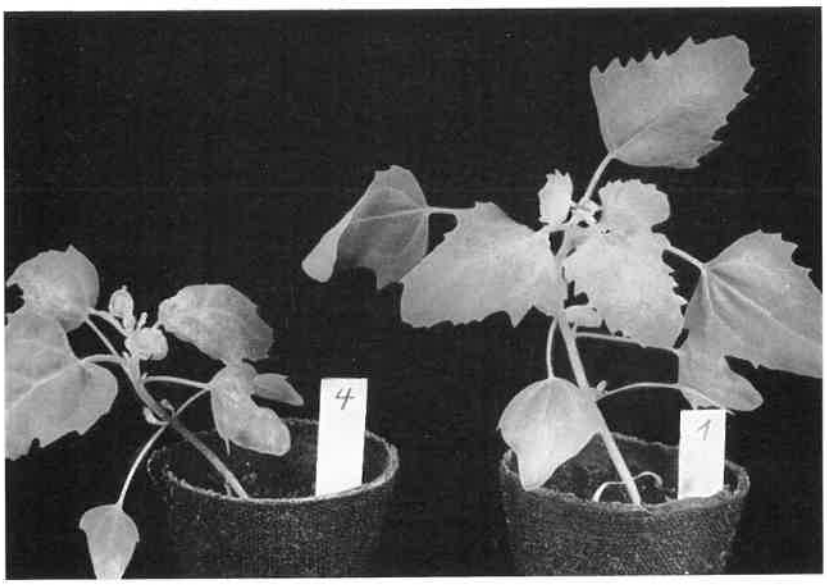

Abb. 1 - *Chenopodium quinoa*, Inokulation mit Extrakt aus reigiskranken Amerikaner-Reben (Isolat Griesheim)
— links: Pressaft + 2,5 % ige wässrige Nikotin-Lösung
— rechts: Pressaft + Phosphatpuffer pH 8
— Inokulation: 25.6.69
— Aufnahme: 5.7.69

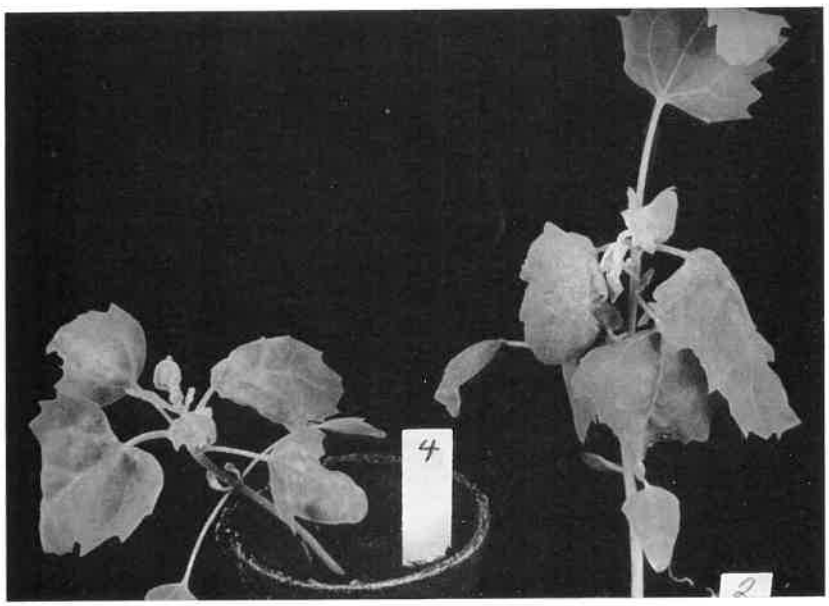

Abb. 2 - *Chenopodium quinoa*, Inokulation mit Extrakt aus reisigkranken Amerikaner-Reben (Isolat Griesheim)
— links: Pressaft + 2,5 % ige wässrige Nikotin-Lösung
— rechts: Pressaft + Boratpuffer pH 9, 0,2 M
— Inokulation: 25.6.69
— Aufnahme: 5.7.69

Tab. 2.

Einfluss verschiedener Virusstabilisatoren auf die Infektiosität

Stabilisator	Konzentration	pH-Wert des Inokulums	Übertragungsrate	Stärke d. Symptome *)
Asparagin	0,1	9,70	100	++
Cystein	0,1	9,23	100	+÷++
Cysteinhydrochlorid	0,1	9,74	100	+÷++
Ephedrin	0,1	9,52	100	+
Hydroxylaminhydrochlorid	0,1	8,10	100	+÷++
Koffein	0,5	8,95	100	+++
Natriumdiäthyldithiocarbamat	0,015 M	9,16	40	(+)
Naphthalinsulfonsäure	0,1	9,49	100	+++
Natriumsulfit	0,5	8,96	100	+
Nikotinsulfat	0,1	8,13	100	+
N'N-Diphenylthioharnstoff	0,015 M	10,67	100	++
Puffer pH 13		8,64	80	+÷++
Nikotin wässrig	2,5	8,66	100	+
Nikotin Puffer pH 13	2,5	9,23	100	+++

*) (+) = Symptomausprägung schwach
\+ = » mittel
++ = » stark
+++ = » sehr stark

Neben diesen Untersuchungen wurden weitere mit Einzelstöcken der Sorte Riesling durchgeführt, die bei Zusatz von Nikotin keinerlei Reaktionen auf den Testpflanzen ergeben hatten. Es wurde Pressaft von 12 Stöcken unter Zusatz von Puffer pH 13, Nikotin pH 9, Xanthin pH 13 und Naphthalinsufonsäure pH 13 hergestellt.

Diese Versuche wurden Anfang August durchgeführt, zu einer Zeit also, in der mechanische Inokulationen besonders mit schwach virulenten Isolaten, zu denen das Riesling-Isolat gehört, keine guten Ergebnisse erbringen. Von den untersuchten Stöcken bewirkten Pressäfte von 4 Stöcken positive Reaktionen auf Testpflanzen mit Puffer, Naphthalinsulfonsäure und Xanthin, während mit Nikotin keinerlei Reaktionen erhalten wurden.

Ebenfalls 100%ige Übertragungsergebnisse in den Monaten August und September konnten mit folgenden Substanzgemischen erzielt werden:

1. Nikotin und Titriplex III (ÄDTA-Äthylendiamintetraessigsäure Dinatriumsalz)
2. Polyäthylenglykol (40 000) in Phosphatpuffer und Ascorbinsäure
3. Gemisch von Na-Diäthyldithiocarbamat, N,N′-Diphenylthioharnstoff und Koffein
4. Mischung von Nikotin, sek. Kaliumphosphat und Cysteinhydrochlorid (= Kirkpatrick-Lindner-Puffer)

In der Kontrolle — Zusatz wässriger Nikotinlösung — lag die Übertragungsrate zwischen 60 und 80%.

3. *Beziehung zwischen Infektiosität der Blattpressäfte und Eiweissextraktion*

Opel und Kegler (1968) fanden bei verschiedenen Obstarten eine enge Korrelation zwischen der Infektiosität von Blattpressäften und der Höhe der Eiweissausbeute, die in gewissen Grenzen als Masstab der Infektiosität angesehen werden kann.

Es erschien interessant, jene Stabilisatoren bzw. Gemische, mit denen gute Übertragungsergebnisse erzielt wurden, auf ihre Fähigkeit zur Eiweissextraktion zu untersuchen. Die photometrische Eiweissbestimmung erfolgte nach Plum, Hermansen und Petersen (1955). Standardextraktionsmittel war das von Schaefer (1969) entwickelte Gemisch aus Tris, Ascorbinsäure, Na-ÄDTA, Dinatriumtetraborat, Natriumchlorid und Polyäthylenglykol 40000.

Da es sich bei diesen Untersuchungen lediglich um orientierende Versuche handelte, soll nicht näher darauf eingegangen werden. Erste Ergebnisse liessen aber erkennen, dass mit den verschiedenen Stabilisierungsgemischen eine unterschiedliche Eiweiss-

ausbeute erreicht wurde und dass ein gewisser Zusammenhang zwischen der Infektiosität und der extrahierbaren Eiweissmenge besteht. Um endgültige Aussagen machen zu können, sind weitere Untersuchungen erforderlich.

SUMMARY

In our studies we were able to obtain an increasing rate of transmission of grape viruses by the choice of suitable stabilisators. The rate of transmission increased also at a time, where usually virus rarely could be transmitted.

Important factors of a successful transmission of grape-viruses are suitable buffer, well defined pH (appr. pH 8) and the addition of certain stabilisators.

An uniform effect of extraction-mixtures seems not to occur, probably because of the different stability of the viruses in various media.

According to the virus-host combination different mixtures of stabilisators are to be used.

In preliminary studies the results of Opel and Kegler with regard to the correlation between infectivity of leaf-extracts and the level of protein-extraction have been confirmed.

RIASSUNTO

Ricerche sull'effetto di sostanze stabilizzanti sulla trasmissione meccanica dei virus della vite.

Attraverso le nostre ricerche abbiamo ottenuto di poter aumentare la trasmissibilità dei virus della vite mediante la scelta di adatte sostanze stabilizzanti, particolarmente in quei casi in cui il virus è difficilmente trasmissibile. Un ruolo importante giocano i tamponi adatti e a pH ben definito (circa pH 8), oltre che l'aggiunta delle sostanze stabilizzanti. Sembra che non si abbia un effetto uniforme delle miscele di estrazione, probabilmente

perchè è diversa la stabilità dei virus a seconda delle miscele con cui si opera. Sono state usate diverse miscele di sostanze stabilizzanti a seconda delle combinazioni virus-pianta ospite. Sono stati confermati i risultati di Opel e Kegler riguardanti il rapporto fra infettività degli estratti fogliari e quantità di proteine estratte.

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HÖHERE BUNDESLEHR - U. VERSUCHSANSTALT FÜR WEIN- UND OBSTBAU
A - 3400 KLOSTERNEUBURG (ÖSTERREICH)

INVESTIGATIONS ON CORRELATIONS BETWEEN PEROXYDASE - ACTIVITY AND VIRUS INFECTION BY CHENOPODIUM MURALE AND VITIS VINIFERA

J. BARNA

Increasing rates of illness through virus infections and the considerable damages resulting from this necessitate the development of methods allowing to detect quickly and reliably vines infected by virus. Based on the known fact of correlations between virus infections and peroxydase-activity, the respective peroxydase-activities (EC I.II.I.7) of healthy and of « reisig-kranker » Vitis vinifera, as well as of infected Chenopodium murale were investigated by means of gelelectrophoretic and spectrophotometric determination of activity. The electrophoretic investigations were carried out by means of vertical-polyacrylamid-gelelectrophoresis in a discontinous system. To demonstrate the peroxydase activities the gels were incubated into 0,1% v/v acetic acid, containing per 100 ml 50 ppm Benzidin as well as 0,2 ml 30% v/v H_2O_2. The spectralphotometric determination of activities of extracts from axillary organs of Chenopodium murale was effected in 0,1 M phosphate buffer (pH value 7,0), using guajacol, at 436 nm. The extract gained from young vine sprouts was investigated by means of benzidin in a 0,1 M acetic buffer (pH value 5,0) at 370 nm. A sharp increase of

peroxydase activity of ill vines could be shown not only by means of polyacrylamidgelelectrophoretic but also by means of spectralphotometric investigations.

RIASSUNTO

Ricerche sulla correlazione fra attività perossidasica ed infezione virale in Chenopodium murale e Vitis vinifera

La diffusione delle malattie virali ed i notevoli danni da queste provocate, ci hanno indotto a studiare nuovi metodi atti ad evidenziare in modo rapido ed inequivocabile le viti infette da virus.

Basandoci sul fatto già noto della correlazione esistente fra attività perossidasica ed infezione virale, si sono studiate le rispettive attività perossidasiche di viti sane e di viti affette da arricciamento, come pure di Chenopodium murale, determinando l'attività perossidasica sia colla tecnica della elettroforesi in gel di poliacrilamide che per via spettrofotometrica.

Gli studi elettroforetici furono eseguiti con l'elettroforesi verticale in gel di poliacrilamide in un sistema discontinuo. Per evidenziare le attività perossidasiche i gel furono incubati in acido acetico allo 0,1% (v/v), contenente 50 ppm di benzidina in 100 ml e 0,2 ml di acqua ossigenata al 30% (v/v). La determinazione spettrofotometrica delle attività degli estratti ottenuti dagli organi ascellari di Chenopodium murale fu fatta in tampone fosfato 0,1 M, pH 7,0, usando guaiacolo, alla lunghezza d'onda di 436 mμ.

L'estratto ottenuto da germogli di giovane vite fu analizzato per mezzo di benzidina in tampone acetato 0,1 M, pH 5,0, alla lunghezza d'onda di 370 mμ.

Un netto aumento dell'attività perossidasica di viti ammalate può essere messo in evidenza non solo con la elettroforesi in gel di poliacrilamide ma anche col metodo spettrofotometrico.

PREVENZIONE E TRATTAMENTI TERAPEUTICI

Disease control

(*chairman*: W.B. HEWITT)

FEDERAL AGRICULTURAL RESEARCH STATION OF LAUSANNE, CH 1260 NYON (SWITZERLAND)

PREVENTION AND CONTROL OF VIRUS AND MYCOPLASMA-LIKE DISEASES OF THE GRAPEVINE

R. BOVEY

The prevention and control of any plant disease largely depends on the knowledge of its etiology and epidemiology. In the case of the grapevine diseases that were considered until five years ago as being caused by viruses, this knowledge varies considerably. It is fairly good for the soil-borne viruses, which include several « Nepoviruses » transmitted by nematodes, and tobacco necrosis virus, transmitted by zoospores of the fungus *Olpidium brassicae,* and for a few well known viruses occasionally found on the grapevine, such as tobacco mosaic virus, sowbane mosaic virus and tomato bushy stunt virus. To a second group belong a few diseases that are no longer considered to be caused by viruses. The « flavescence dorée », the « bois noir », and possibly also corky bark, are probably caused by mycoplasma-like microorganisms (Caudwell *et al.,* 1971 b), whereas Pierce's disease seems to be due to rickettsia-like bacteria (Hopkins and Mollenhauer, 1973). The remaining « virus diseases » form a third group where the only proof that a virus is the causal agent of the disease is the fact that they are graft transmissible and that no other pathogen has been found constantly associated with them. The recent history of phytopathology as shown how this can be misleading. It would be better to call them « virus-

like diseases ». Litte or nothing is known about their transmission, and their control is based only on providing healthy propagation material.

PREVENTION AND CONTROL OF VIRUS DISEASES OF THE GRAPEVINE

As there is no treatment for curing a virus disease in the field, control measures are mainly preventive. They can be summarized as follows:

1) Obtaining healthy planting material.
2) Cultivating the new plantings in a soil that is free of vectors of soil borne viruses.
3) Avoiding contamination of the vineyards after planting.
4) Breeding vector resistant rootstocks which would permit replanting in infested soil. This would be the best solution for the control of soil-borne viruses.

Obtaining healthy material: New varieties obtained as seedlings are usually free of viruses and should be protected from contamination by soil- or airborne viruses. Old varieties are often infected by one or several viruses, which may cause very variable symptoms, or no symptoms at all, according to the type of viruses that are present.

Visual selection can give quite good results with little cost in a rather short time, but it needs a good knowledge of the symptoms of the virus diseases and of some other diseases or pests that can cause confusion with virus symptoms. It has a limited scope because of the latent viruses or infections by mild strains. The examination of *trabeculae* in sections of stems is useful on american rootstocks, where they develop abundantly when these plants are infected by fanleaf virus, but this test is not sensitive enough with *vinifera* varieties because the number of trabeculae that are formed is much lower.

Indexing increases the sensitivity and precision of the detection of the virus infections, but it implies also a visual appreciation, with the same difficulty as in the case of visual selection: where is the limit between healthy and infected plants? The

various methods of indexing are discussed in another paper of this meeting. For the selection purposes, both herbaceous hosts and *Vitis* hosts are useful.

Serology is used increasingly for detection and identification of grapevine viruses. The agar double-diffusion method of Ouchterlony can be used with grapevine leaf extracts, provided the virus is concentrated by centrifugation (Vuittenez *et al.*, 1964; Vuittenez, 1967). The latex method is more sensitive and can be used with crude sap of grapevine leaves, clarified by centrifugation (Vuittenez et Kuszala, 1972). Bercks (1972) considers serology as a valuable method for detecting infection in glasshouse-grown cuttings but not for grapevine leaves taken in the vineyard.

Thermotherapy or heat treatment provides a very useful method for obtaining healthy material from virus-infected plants. It is particularly valuable when varieties are so heavily infected that it is impossible or very difficult to find healthy plants in the vineyards. Two different methods are now in use. The first has been developed at the Plant pathology department of the University of California at Davis (Goheen *et al.*, 1966). Potted plants are cultivated 1-3 moths or more in a special room at 38°C, and the tips of the shoots grown during this period are rooted under mist in a glasshouse.

The second method has been devised in France by Mrs Galzy (1964, 1966, 1969 a, 1969 b, 1970). Cuttings of green shoots about 2-3 cm long with a bud at their superior extremity are grown aseptically in glass tubes containing agar with a nutrient solution. After rooting at 20°C, the plants are maintained for 3 months at 35°C in a special incubator lighted by fluorescent tubes. After treatment, they are potted and grown in a glasshouse.

The following viruses have been eliminated by heat treatment: Fanleaf (including yellow mosaic and veinbanding): several authors.

Yellow vein, asteroid mosaic and corky bark: Goheen *et al.*, 1966; Nyland and Goheen, 1969.

Meristem culture has been used by Goheen *et al.* (1966) and Wolfswinkel (1966) in the hope of curing infected grapevines, but this method gave little success. A review on therapeutic methods applied to the grapevine has been published recently by Bovey (1972).

Cultivating in healthy soil: control of nematodes that transmit grapevine viruses has been achieved by two means: crop rotation (or fallowing), and soil disinfection.

Crop rotation is likely to give good results with fanleaf and *Xiphinema index*, as this nematode has a narrow host range, but there are few experiments on this method. Vuittenez (1970) recommends a fallowing period of ten years before replanting in infested soil. Dalmasso *et al.* (1972) show that in the south of France the population of *Xiphinema index* disappears almost entirely within two years after pulling out the old vines, provided the eradication has been made properly. Dalmasso (1971) recommends cultivating lupin, lucerne and cereals during 7 years before replanting. In California, Raski *et al.* (1965) have shown that both root pieces and *Xiphinema index* could be found living in the soil 4.5 years after the vines were removed, and conclude that a period of at least 5 years is necessary before replanting a new vineyard in infested soil.

Soil disinfection with fumigant nematicides provides a quicker way of eliminating the nematode vectors. The first experiments have been made in Burgundy by Vuittenez (1958, 1960, 1961). DD, a mixture (50/50) of dichloropropane and dichloropropene distributed in the soil at 20 cm and at 1140 kg/ha, and a mixture of methyl bromide, carbon tetrachloride and dichlorethane (379 kg/ha pure methyl bromide) both gave a good control of the nematode population and of fanleaf. Carbon disulfide at 3020 kg/ha was less successful. Similar experiments were made recently in the south of France (Boubals *et al.*, 1965; Boubals and Dalmasso, 1968 a, 1968 b).

In a recent review of this problem, Dalmasso (1971) recommends following doses:

	For immediate replanting	For replanting after 3 years
— DD (Dichloropropane- dichloropropene 50/50)	1000 l/ha	750 l/ha
— Telone (Dichloropropene)	600 l/ha	500 l/ha
— Dibromoethane (mixed 50/50 with dichloropropane)	800 l/ha	600 l/ha

In California, fumigation at a depth of 20-25 cm has not given good results, because both vector and fanleaf virus can be found living as deep as 2.40 m and probably deeper. RASKI *et al.* (1971) obtained a good control of fanleaf and *Xiphinema index* using 1,3-dichloropropene at 200 gallons per acre (1870 l/ha) at a depth of 75-90 cm and a second treatment with 50 gallons per acre (467 l/ha) at a depth of 20-25 cm. Carbon disulfide and methyl bromide in shallow applications gave no satisfactory results. Soil fumigation with DD stimulates the growth of the newly planted grapevines (VUITTENEZ, 1960; RIVES and LECLAIR, 1966; BRÜCKBAUER, 1969) but the real causes of this stimulation are not yet clearly understood. BOUBALS and DALMASSO (1968 a, 1968 b) noticed an important increase of *Paratylenchus* sp. in plots treated with DD, whereas this genus was almost absent in control plots. Soil disinfection should be made only in vineyards where nematode vectors are present.

Avoiding contamination of the vineyards after planting: There is little experience on this point. Care should be taken to avoid bringing nematodes from infested adjacent vineyards with machines, or with soil. The risk of transportation of *Xiphinema* with surface water is probably low, as these nematodes are not present in the upper layer of soil.

Breeding for resistance: The best answer to the problem of the control of fanleaf and other nepoviruses in viticulture would be a rootstock that is a bad host for the vectors, or totally resistant.

PREVENTION AND CONTROL OF MYCOPLASMA AND RICKETTSIA-LIKE DISEASES OF THE GRAPEVINE

In the south-west of France, the flavescence dorée, caused by mycoplasma-like microorganisms (CAUDWELL *et al.*, 1971 b) transmitted by the leafhopper *Scaphoideus littoralis* Ball. (SCHVESTER *et al.*, 1963), has been controlled without difficulty by three insecticide sprayings against the larval stages, provided they are made on a large area. The disappearance of the vectors results in the disappearance of the symptoms, because of the recovery of infected vines (CAUDWELL, 1969; CAUDWELL anod SCHVESTER, 1970). No control measure has been found against the endemic form of the

flavescence dorée, which Caudwell proposes now to call «Bois noir» disease and wich is not transmitted by *Scaphoideus littoralis* (Caudwell *et al.*, 1971 a).

Pierce's disease, which now seems to be caused by rickettsia-like microorganisms (Hopkins and Mollenhauer, 1973) is transmitted by several species of leafhoppers and has a very large host range. So far, no control method has been proposed (Hewitt, 1970).

DISCUSSION

With visual selection alone, it has been possible to raise the productivity by 20-85% without loss in quality (Bovey *et al.*, 1967). Indexing and serology have permitted obtaining clones of many varieties that are free of most or all of the known grapevine viruses. A list of such material obtained in several Institutes and available for research purposes is published by the I.C.V.G. Most interesting is the fact that heat treatment seems to increase the yield of clones that were considered virus-free on the basis of indexing on herbaceous and *Vitis* hosts.

The virus-tested material is now being multiplied and tried in several countries. It is likely to be distributed on a large scale within the next twenty years. What consequences can be expected from the use of this new healthier material? The considerable increase in productivity that will certainly occur will be of course advantageous for the viticulturists, and have economic consequences that will not be discussed here. There are also some risks which I shall briefly mention:

— *Overproduction*: It is well known that the yeild of grapevine cannot be increased beyond a limit without a loss in the quality of the grapes and the wine. Whenever the quality is an important factor, it will be necessary to adapt the method of cultivation to the greater productivity of the new material so that the best combination between quality and quantity is obtained. In selecting among virus-tested clones, a great emphasis should be given to the criteria of quality.

— *Sensitivity to Botrytis*: The very vigourous vines with compact grapes obtained with the virus-tested matérial are much more sensitive to *Botrytis cinerea* than the usual non-selected vines.

Selecting for resistance or for a lower sensitivity to this fungus would be useful.

— *Loss of genetical variability*: There is a danger of losing a valuable genetical variability by choosing a small number of clones for each variety. It would be useful to keep samples of each variety with its present degree of variation, as suggested by Huglin (1972).

— *Possible role of cross protection*: It is very unlikely that all plantings with the new virus-tested material will be made in healthy soil, free of infected nematodes. It is known that the symptoms of many virus diseases are more severe shortly after the infection (shock symptoms) than after some time, and a mild strain can protect the plant against the infection by a severe strain of the same virus (cross protection). What will happen when « virus-free » vines are planted in contaminated soil? Will they be more severely struck by the virus than vines already infected by a mild strain of fanleaf? It would be interesting to gain some experience about this point before planting the selected material on large areas, if the soil is not quite pathogen free.

— *Alterations due to the heat treatment*: Mur *et al.* (1972) mention changes in pilosity and leaf shape after heat treatment of several varieties, in comparison with the original untreated material: deeper sinuses, more pubescent leaves on the upper side, reddish colour of the shoots, bronze colour of the young leaves. They suggest that this could be a mutation.

In conclusion, we can say that the new material obtained by selection and/or by heat treatment will undoubtedly increase the productivity of the viticulture.

In future years, it will probably make most of the difference between a profitable and a non profitable vineyard. However, it is highly desirable, especially with the heat treated clones, that thorough experimentation be carried out under all conditions.

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RIASSUNTO

Prevenzione e lotta contro le malattie della vite dovute a virus o a microrganismi riferibili a micoplasmi

Essendo impossibile curare le malattie da virus in pieno campo, i mezzi di difesa sono essenzialmente preventivi e possono essere riassunti come segue:

1) ottenimento di materiale da propagazione sano;
2) coltivazione dei nuovi impianti in terreni esenti da vettori di virus;
3) difesa dei vigneti da infezioni successive all'impianto;
4) selezione di portinnesti resistenti ai vettori.

Per quanto riguarda la Flavescenza dorata (malattia che si ritiene dovuta a micoplasmi), si ottiene una soddisfacente prevenzione mediante trattamenti insetticidi contro il vettore (*Scaphoideus littoralis*).

Sono infine discussi alcuni problemi che potrebbero sorgere con l'impiego su vasta scala di materiale virus-esente.

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LANDES- LEHR- UND VERSUCHSANSTALT FUER WEINBAU, GARTENBAU UND LANDWIRTSCHAFT
ZENTRALSTELLE FUER KLONENSELEKTION - TRIER

DIE ERHALTUNGSZUECHTUNG ALTER *VITIS VINIFERA*-SORTEN IN DEUTSCHLAND

H. SCHOEFFLING und K.H. FAAS

A. ALLGEMEINES UND GESCHICHTLICHE ENTWICKLUNG

Rebersorten koennen in ihrer Leistungsfaehigkeit nachlassen, wenn sie nicht mit Hilfe der Erhaltungszuechtung, d. h. der fortwaehrenden Auslese und Vermehrung der leistungsfaehigsten Stoecke auf der groesstmoeglichen Leistungshoehe gehalten werden. Sinn und Zweck der Erhaltungszuechtung ist es also, die Leistungsfaehigkeit einer Sorte zu erhalten. Bei der Selektion der Einzelstoecke koennen mehrere Verfahren angewendet werden:

1. Negative Massenauslese
2. Positive Massenauslese
3. Individual- oder Einzelstockauslese mit Nachkommenschaftspruefung, auch Klonenselektion genannt.

Zu 1.: Dieses Verfahren, naemlich die Vermehrung einer Population unter Ausscheidung der leistungsschwachen Stoecke, genuegt den Anforderungen der Erhaltungszuechtung nicht.

Zu 2.: Die Vermehrung aller positiv beurteilten Stoecke beansprucht mehr Zuchtstufen, um zu leistungsfaehigen Bestaenden zu gelangen. Unter bestimmten Bedingungen kann

dieses Verfahren zur befriedigenden Erhaltung einer Sorte fuehren.

Zu 3.: Positiv bewertete Einzelstoecke werden getrennt vermehrt. Hierbei stehen die Nachkommen dieser Stoecke (Klone) zusammen und werden als Gesamtheit beobachtet und geprueft. Klone, welche dem Klonendurchschnitt nicht entsprechen, werden eliminiert. Nur durch dieses Verfahren kann der Erhaltungszuechter die wirklich wertvollen Klone herausfinden.

In Deutschland begann die Erhaltungszuechtung alter *Vitis vinifera*-Sorten auf der Basis der Klonenselektion im Jahre 1876. Das Weingut Froelich in Edenkoben (Pfalz) bearbeitete damals 250 Klone der Sorte Silvaner. Die leistungsfaehigsten Klone wurden im Jahre 1921 als Vermehrungsanlage klonaler Selektion staatlich anerkannt. Im Jahre 1925 folgte ihre Eintragung als Hochzucht-Pflanzgut in das DLG-Register in Berlin.

Weitere Stationen in der Klonenselektion waren:

— 1900 Beginn der Klonenselektion an der Mosel
— 1907 » » » in Wuerttemberg
— 1909 » » » in Rheinhessen
— 1910 » » » in Rheingau
— 1918 » » » in Baden
— 1921 » » » an der Nahe
— 1925 » » » in Bayern
— 1932 » » » an der Ahr.

Mit dem Inkrafttreten des *Gesetzes ueber Sortenschutz und Saatgut von Kulturpflanzen* aus dem Jahre 1953 wurden die Erhaltungszuechter, soweit ihre Klone den gesetzlichen Bestimmungen entsprachen, in das Besondere Sortenverzeichnis beim Bundessortenamt aufgenommen.

Es folgte im gleichen Jahre die *Verordnung ueber Zulassung von Handels- und Importsaatgut* sowie im Jahre 1954 die *Verordnung ueber die staatliche Anerkennung von Pflanzgut.*

Seit dieser Zeit kann nur noch Stammpflanzgut staatlich anerkannt werden.

Stammpflanzgut muss nachweislich erhaltungszuechterisch bearbeitet sein. In Ausnahmefaellen duerfen Populationen als Handelspflanzgut zugelassen werden.

Waehrend bei « zugelassenem Pflanzgut » noch 5 v.H. der Stoecke uebertragbare Abbaukrankheiten haben durften, lag dieser Prozentsatz beim « anerkannten Klonenpflanzgut » bei 1 v.H.

Die abbaukranken Stoecke mussten allerdings spaetestens bei der Besichtigung der Vermehrungsanlage entfernt werden.

Im *Gesetz ueber den Verkehr mit Saatgut* (SVG) aus dem Jahre 1968, in Verbindung mit der *Verordnung ueber Pflanzgut von Ertragsreben und Unterlagsreben*, wurden die bisherigen Bestimmungen zur Erhaltungszuechtung und Klonenselektion neu gefasst, verbessert und insbesondere bezueglich der sanitaeren Aspekte ergaenzt und verschaerft.

Einmal ist die Klassifizierung des Klonenpflanzgutes in Basis- und Zertifiziertes Pflanzgut einschnieded. Danach gelangt beim Winzer nur noch die 3. Vermehrungsstufe zum Anbau. Frueher konnte es die 4., 5., 6. usw. sein! Zum anderen wird festgelegt, dass die Vermehrungsflaechen zum Zeitpunkt der Pflanzung der Reben nicht von Viren befallen sein duerfen. Rebenbestaende, deren Pflanzgut Symptome von Viruskrankheiten zeigen, muessen von der Rebenanerkennung ausgeschlossen werden.

Aber auch der Begriff « Erhaltungszuechter » wird definiert. Nur wer waehrend der letzeten 3 Zuchtstufen (s. § 63 SVG) eine Sorte nach den Grundsaetzen systematischer Erhaltungszuechtung bearbeitet hat, kann als Erhaltungszuechter in die Sortenliste, die das Besondere Sortenverzeichnis abloest, eingetragen werden.

Nach § 73 SVG sind die eingetragenen Klone vom Bundessortenamt staendig auf Homogenitaet und Bestaendigkeit ihrer Leistungen zu ueberwachen. So kann die Eintragung eines Erhaltungszuechters geloescht werden, wenn die Sorte nicht mehr nach den Grundsaetzen systematischer Erhaltungszuechtung bearbeitet wird (Ueberwachungspruefung).

Nach dem derzeitigen Stand sind in Deutschland 37 Erhaltungszuechter in die Sortenliste beim Bundessortenamt eingetragen. Diese Zuechter bearbeiten insgesamt 20 Sorten und 259 Klone.

In Tabelle 1 sind die in der Sortenliste gefuehrten Sorten, ihre Erhaltungszuechter und ihre Klone zusammengestellt.

Tabelle 1.

Sorten, Erhaltungszuechter und Klone laut Sortenliste 1973.

Lfd. Nr.	Sorten	Erhaltungs-zuechter	Klone
1	Weisser Riesling	15	61
2	Gruener Silvaner	15	55
3	Mueller-Thurgau	7	32
4	Blauer Spaetburgunder	6	16
5	Rulaender	4	19
6	Blauer Portugieser	3	13
7	Roter Traminer	2	8
8	Weisser Gutedel	2	9
9	Weisser Burgunder	2	8
10	Blauer Fruehburgunder	2	7
11	Roter Gutedel	1	1
12	Gelber Muskateller	1	1
13	Auxerrois	1	3
14	Muskat Ottonel	1	2
15	Weisser Elbling	1	1
16	Roter Elbling	1	1
17	Blauer Trollinger	1	3
18	Blauer Limberger	1	3
19	Frueher roter Malvasier	1	15
20	Muellerrebe	1	1

20 Sorten werden von 68 Erhaltungszuechtern mit 259 Klonen bearbeitet, wobei die Zahl 68 durch die getrennte Ausweisung fuer jede Rebsorte bedingt ist.

Bemerkenswert ist, dass es in Deutschland Klone alter *Vitis vinifera*-Sorten gibt, die bereits seit Jahrzehnten zuechterisch bearbeitet werden und dass von den derzeitig 37 verschiedenen deutschen Erhaltungszuechtern 20 private und 17 staatliche sind. Das entspricht einem Verhaeltnis von 54 v.H. zu 46 v.H.

Geht man von den registrierten Klonen aus, so werden 170=65 v.H. privatwirtschaftlich betreut. Nur 89=35 v.H. sind in staatlicher Hand.

In Deutschland ueberwiegt somit die private Klonenzuechtung.

Auf die Arbeit der privaten Klonenzuechter und die parallel hierzu verbesserte Duengung und Schaedlingsbekaempfung sind die in den letzten Jahrzehnten staendig ansteigenden Ertraege im deutschen Weinbau zurueckzufuehren (s. Tabelle 2).

TABELLE 2.

Ertragsentwicklung im deutschen Weinbau

Merkmal	1900	1970
Traubenertrag hl/ha	10 - 20	80 - 100

B. LEISTUNGSPRUEFUNGEN IM RAHMEN DER ERHALTUNGSZUECHTUNG

Im Rahmen der Erhaltungszuechtung muessen die Klone Leistungspruefungen unterzogen werden. Diese Leistungspruefungen werden im Zuchtbetrieb durchgefuehrt. Sie zeigen immer wieder, dass durch Individual- oder Einzelstock- auslese mit Nachkommenschaftspruefung (Klonenselektion) die grossten Ertragssteigerungen zu erreichen sind.

Darueberhinaus wird zuechterisch bearbeitetes Pflanzgut untereinander verglichen. Diese Vergleichspruefungen werden von staatlichen Instituten durchgefuehrt.

Erhaltungszuechter, deren Zuchtmaterial weit unter dem Leistungsdurchschnitt liegt, muessen damit rechnen, in der Sortenliste gestrichen zu werden. Zwei derartige Faelle gab es in den Jahren 1972/1973, wo § 73 SVG zur Anwendung kam.

C. GESUNDAUFBAU IM RAHMEN DER ERHALTUNGSZUECHTUNG

Langjaehrig in obigem Sinne zuechterisch bearbeitete Klone von *Vitis vinifera*-Sorten sind in den hohen Anbaustufen erfahrungsgemaess weitgehend frei von schweren Formen der Rollkrankheit und der Reisigkrankheit.

Im Laufe ihrer Vermehrung werden sie aber immer wieder auf Unterlagen gepfropft, die u. U. auch «zuechterisch bearbeitet» sind, deren zuechterische Bearbeitung aber nur in sehr eingeschraenktem Masse die Gewaehr fuer dass Freisein von den genannten Virosen bietet. Das ist in erster Linie darin begruendet, dass Leistungspruefungen auf der Grundlage weinbaulicher Ertraege bei Amerikanerreben nicht durchgefuehrt werden koennen. Deshalb kann es vorkommen, dass die verwendeten Unterlagen den Verseuchungsgrad des Edelreis- Klonenmaterials vergroessern, wodurch der Endverbraucher von Rebenpflanzgut nicht oder nur in geringem Masse an der Leistungsfaehigkeit der Klone partizipiert. Aus diesem Grunde muss die Erhaltungszuechtung bei Amerikanerreben voellig neu ueberdacht werden.

Die z. Z. gueltigen EWG-Richtlinien zur Erzeugung von Rebenpflanzgut schreiben vor: *Rebmutterbestaende zur Erzeugung von Basis-Pflanzgut sind von den Erregern der Reisigkrankheit und der Rollkrankheit freizuhalten; Mutterrebenbestaende zur Erzeugung von Zertifiziertem Pflanzgut sind freizuhalten von Pflanzen, die Symptome einer der genannten Virosen aufweisen.*

Ein Klone-Neuaufbau von *Vitis vinifera*-Sorten muss nach diesen Richtlinien folgendermassen vonstatten gehen:

Ausgangsstufe

1973 Es werden etwa 20 besonders leistungsfaehige Stoecke nach etwa 3jaehriger Beobachtung ausgewertet.

1976 Die 20 Ausgangsstoecke werden stockgetrennt auf 10 Pflanzen je Ausgangsstock vermehrt:
a. wurzelecht oder
b. auf virologisch getestete Unterlagen gepfropft.

Erste Vermehrungsstufe

1976 Die 20 Vermehrungen à 10 Stock werden jeweils in 2 Wiederholungen à 5 Pflanzen in ein entseuchtes Gelaende ausgepflanzt.

1978 Durchfuehrung von Leistungspruefungen (3 Jahre) sowie Beginn einer 3jaehrigen virologischen Untersuchung.

1981 Auswahl derjenigen Einheiten, welche im Virustest negativen Befund zeigten und auch in ihrer Leistung befriedigten.

1982 Die der virologisch gepruefteten Wiederholung entstammenden 5er Gruppen werden infektionsgeschuetzt weitervermehrt:
a. wurzelecht oder
b. auf virologisch getestete Unterlagen gepfropft.

Zweite Vermehrungsstufe

1982 Die Anpflanzung dieser « Vorstufen » erfolgt in ein entseuchtes Feld als Mutterrebenbestand zur Erzeugung von Basis-Pflanzgut.

Die Notwendigkeit der Einbeziehung besonderer sanitaerer Massnahmen in die Erhaltungszuechtung ist nicht nur angesichts der vorgesehenen EWG-Bestimmungen gegeben, sondern auch deswegen, weil das *Bundessortenamt* bei der Eintragung einer Sorte bzw. eines Klones kuenftig ein *amtliches Zeugnis* ueber das Freisein von Viren verlangen wird.

Aber auch im Rahmen der *Rebenanenerkennung* muss gemaess den geplanten Rechtsvorschriften zum Saatgutgesetz demnaechst der *Nachweis* erbracht werden, dass die Mutterrebenbestaende zur Erzeugung von Basis-Pflanzgut virologisch bearbeitet sind.

Mit der Arbeit in der beschriebenen Form haben fast alle deutschen Erhaltungszuechter begonnen, so dass von allen Klonen spaetestens im Jahre 1980 Vermehrungsanlagen zur Gewinnung von gesundem Basis-Pflanzgut vorhanden sein duerften, die den EWG-Richtlinien entsprechen.

D. PROBLEME DER PRIVATWIRTSCHAFTLICHEN ERHALTUNGSZUECHTUNG

Das Land Rheinland-Pfalz stellt als groesstes weinbautreibendes Land der BRD 70 v.H. der eingetragenen Erhaltungszuechter (26 von 37) und 45 v.H. aller registrierten Klone (114 von 259).

Hinzu kommt, dass von den eingetragenen Erhaltungszuechtern in Rheinland-Pfalz 60 v.H. (16 von 20) private sind; 60 v.H. (81 von 130) der registrierten Klonenzahl werden privatwirtschaftlich betreut.

Inzwischen sind aber in Rheinland-Pfalz weitere Zuechter mit erfolgversprechenden Selektionen zu finden.

Damit ist die besondere Situation der ueberwiegend auf privatwirtschaftlicher Basis stehenden Klonenselektion in Deutschland gekennzeichnet.

Es stellt sich die Frage, ob und inwieweit die privaten Erhaltungszuechter der alten *Vitis vinifera*-Sorten in Anbetracht der zu erwartenden steigenden finanziellen Belastungen durch Virus-Tests und Bodenentseuchung weiterarbeiten koennen. Da es keinerlei staatliche Zuschuesse gibt, auf der anderen Seite hoeher Unkosten nicht voll im Edelreiserpreis weitergegebn werden koennen, duerfte in der Zukunft die wirtschaftliche Basis der privaten Erhaltungszuechter stark belastet werden.

Schon jetzt zeigt sich eine Reduzierung der Klonenzahl. Dies ist u.E. aber nicht unbedingt als Nachteil zu werten. Einmal werden auf diese Weise nur die leistungsfaehigsten Klone beibehalten, andererseits kann die Erhaltungszuechtung intensiviert werden.

Dennoch waere ein voelliges Ausschalten der privaten Erhaltungszuechter nicht zu begruessen, weil die Klonenselektion bei ihnen genauso erfolgreich ist wie in den staatlichen Betrieben, was die Geschichte gezeigt hat.

Eine Stuetze fuer die privaten Erhaltungszuechter (aber auch fuer die staatlichen Stellen) in Rheinland-Pfalz soll die Zentralstelle fuer Klonenselektion in Trier sein.

Ihre Aufgabe ist es, durch Herausgabe von Richtlinien

1. fuer das Versuchswesen
2. fuer die klonale Selektion und
3. fuer die Viruskontrolle in Vermehrungsanlagen

Die zahlreichen in Deutschland bereits jetzt vorhandenen hochleistungsfaehigen Klone werden erhalten werden muessen. Die den Erhaltungszuechtern mit fachlichem Rat zur Seite zu stehen. Erhaltung ihrer Leistungsfaehigkeit wird weiterhin zum grossen Teil den privaten Erhaltungszuechtern obliegen, denn auf ihre Arbeit kann und soll nicht verzichtet werden.

In diesem Zusammenhang stellt sich die vordringliche Aufgabe, die EWG-Richtlinien in die deutsche Gesetzgebung so einzubeziehen,

dass sowohl den EWG-Partnern als auch der besonderen Situation der heimischen Erhaltungszuechtung — die durch zahlreiche, jahrzehntelang bearbeitete Klone in privater Hand gekennzeichnet ist — Rechnung getragen wird. Insbesondere wird die Verkehrsfaehigkeit deutschen Rebenpflanzgutes alter *Vitis vinifera*-Sorten innerhalb der EWG *fuer eine Uebergangszeit* nicht von nachweislich durchgefuehrten Virus-Tests abhaengen duerfen. Vielmehr sollte der Nachweis klonaler Selektion unter *Einbeziehung von Leistungspruefungen* hierfuer massgbend sein.

SUMMARY

Clonal-selection of grapes in Germany originated in the region of Palatinate in 1876. The private situation of clonal selection is more important than the official one.

The first time a clone was officially registered, was in the list of the German Agriculture Society in 1925 at Berlin. In 1953 all clone-breeders were registered in the Special List of Plants and in 1968 in the List of Plants of the Federal Office of Plants.

For the first time, vines were officially acknowledged in 1921. In 1954 a new order of acknowledgment was edited. In 1968 there originated new classifications: preliminary, base and certified plants. Since then, virus-diseases are not allowed.

A clone-breeder, according to § 63 of the law of plant-circulation, is a man who works in accordance with the principles of systematic selection (3 steps of multiplication: preliminary, intermediate and principal selection). This is also necessary for the inscription in the List of Plants.

The inscription in the List of Plants is in force for 20 years. A prolongation of inscription is only possible, when the production of the clone has been improved soundly. If the results are not sufficient, the clone-breeders will be eliminated from the List of Plants.

The production of healthy material is very expensive for every clone-breeder. At present they have no financial governmental aid.

The Central Station for Clonal-selection must provide help to the clone-breeders during their work, particularly in virus control and for research.

RIASSUNTO

La selezione clonale delle varietà di Vitis vinifera *in Germania*

La selezione clonale della vite fu iniziata in Germania nel 1876 nella regione del Palatinato. Le collezioni private di materiale selezionato sono più importanti di quelle allestite da enti pubblici.

La prima registrazione ufficiale di un clone avvenne nel 1925 nei registri della Società Agricola Germanica a Berlino. Nel 1953 tutti i selezionatori furono iscritti in uno speciale registro delle varietà e nel 1968 nel Registro Federale delle Varietà.

Un primo riconoscimento ufficiale del materiale selezionato si ebbe nel 1921. Seguirono nuove norme nel 1954. Nel 1968 fu stabilita l'attuale classificazione del materiale, indicato come: preliminare, base e certificato. Da allora non è più tollerata la presenza di virosi.

Secondo l'articolo 63 della legge sulla produzione e il commercio del materiale da propagazione, è selezionatore colui che effettua la selezione secondo 3 tappe fondamentali: preliminare, intermedia e principale. Questo requisito è necessario anche per l'iscrizione nel Registro delle Varietà.

L'iscrizione nel Registro delle Varietà è valida per 20 anni; può essere prolungata soltanto se il clone ha dimostrato, nei prescritti controlli, caratteristiche di produzione superiori a determinati limiti. In caso contrario il clone viene depennato dal Registro.

La selezione e moltiplicazione del materiale sano è indubbiamente costosa. Attualmente i selezionatori non ricevono alcun finanziamento dallo Stato.

Dovrebbe essere compito dei Centri di Selezione, organizzati da enti pubblici, facilitare l'opera dei selezionatori privati soprattutto nel settore della sperimentazione e del controllo delle virosi.

BIOLOGISCHE BUNDESANSTALT, INSTITUT FÜR REBENKRANKHEITEN, BERNKASTEL

NOTES ON A MODIFICATION IN THE TECHNIQUE FOR INACTIVATING NEPO-VIRUSES IN GRAPES BY HEAT TREATMENT

G. STELLMACH

In 1969, NYLAND and GOHEEN have made an excellent review of the present state of heat therapy of virus diseases of perennial plants. According to that, heat treatments have been employed for ensuring virus freedom in mother plants used for the production of foundation stocks and should be adopted as standard procedure for the movement of plants through quarantines.

The survival of infected plants in the heat chamber is affected by temperature, plant age, length of time since transplanting into containers and seasonal effects. Plants well established in the containers survived better than those recently transplanted. Many candidate vines have been held for periods 1 to 2 years or longer in containers before heat treating.

It is generally recognized, that a treated plant becomes totally infected again with time after it is returned to normal growing temperature. Whithin the plants there may be centers where virus still remains. With relatively prolonged treatment only, complete cure is possible. A whole plant of *Vitis rupestris* cv. du Lot (St. George) was cured of the fanleaf disease and the grapevine fanleaf

virus eliminated from the plant by thermotherapy for 163 days at 38°C (Rives, 1970).

The most plausible hypothesis for the mechanism of heat therapy is that high temperatures cause the destruction of essential chemical activities in both virus and host but the host is better able to recover from the damage.

We have assumed that the destruction of chemical activities caused by high temperatures should be more effective in young green grape tissues than in woody ones. Heat treatment of green grape shoots may be possible under mist. Large cuttings with considerable leaf area can be treated.

MATERIAL AND METHODS

Softwood heat treatment of vines under mist is carried out during June, July and August, with the main batch treated in July. In June, the cutting material may still be a little immature, while treatment in August may give a very quick lignifying of the cuttings. The shoots used for softwood heat treatment may be obtained from fruiting vines which at this time of the year often thinned to give more light to the grapes. Ideally, the shoots used for softwood heat treatment should be short-jointed and well developed, but not yet lignified. The very soft tips of the shoots are usually discarded. The cutting which is of the nodal type, needs to consists of three internodes with four well developed buds.

In heat treating cuttings under mist, it is essential that a well-drained growing medium be used and the bed raised, equipped with drainage tiles, or otherwise provided for adequate removal of any excess water.

Looking for the best growing medium, it was found in trials that the highest percentage of rooted and growing cuttings was obtained in gravel of vulcanic ashes. Using the familiar red clay flower pots the cuttings may be inserted with three buds above the growing medium.

For inactivation studies with fanleaf and raspberry-ringspot viruses, infected indicator vines of the variety « Siegfried-Rebe » (FS-4-201-39) are used.

Thus the final plants usually do not need to be re-indexed. Other candidate vines for heat treatment are from many sources.

Vines of different varieties known to be infected by NEPO-viruses have been treated. Green tips or leaves from the candidate are taken for mechanical inoculation of expressed sap to *Chenopodium quinoa* or *C. murale*. To concentrate the virus ultracentrifugation has been employed. Usually 2 or 3 cycles are used involving low-speed centrifugation to remove contaminants. Some sort of reducing agents are included in the buffer used during maceration of leaves. *Chenopodium* indicators are held at least 30 days (Stellmach, 1969).

Intermittent-mist water sprays over the growing bed have been found very effective in providing a film of water over the leaves which reduces transpiration and respiration. So the leaves do not wilt. Such sprays lowers the temperature of the leaves. Therefore, in our equipment the water spray is heated.

In a mist installation especially the heat treatment type damage to the cuttings will result if the leaves are allowed to become dry for very long. Even 30 minutes without water can be disastrous. An electrically operated timer mechanism is available wich will operate the mist as desired. This most successful type operates a tank-pump-nozzle-system to produce an intermittent mist at any desired combination of timing intervals such as 15 seconds ON and 20 minutes OFF. This type of control mechanism is relatively foolproof, and although it does not automatically compensate for variations in humidity conditions, it can be adjusted closely enough to give satisfactory results.

The cuttings inserted in clay pots with vulcanic ashes as the growing medium are placed above a hotbed. Heat is provided artificially below the pots by electric heating cables. The hotbed is placed in a large glass box established in the greenhouse. Automatic temperature control can be obtained with a thermostat. At night the cabinet operates over a temperature range of 35-36°C, during the daylight hours over a temperature range of 36-39°C.

A second type of growing chamber is used. This one operates under continuous artificial light and the air temperature is controlled at 30°C within ± 0,5°C. A continuously operating fan circulates air through the cabinet and conditioning equipment at a sufficiently high rate to ensure even temperature distribution in the working space. The chamber is equipped with a mist system too (Fig. 1).

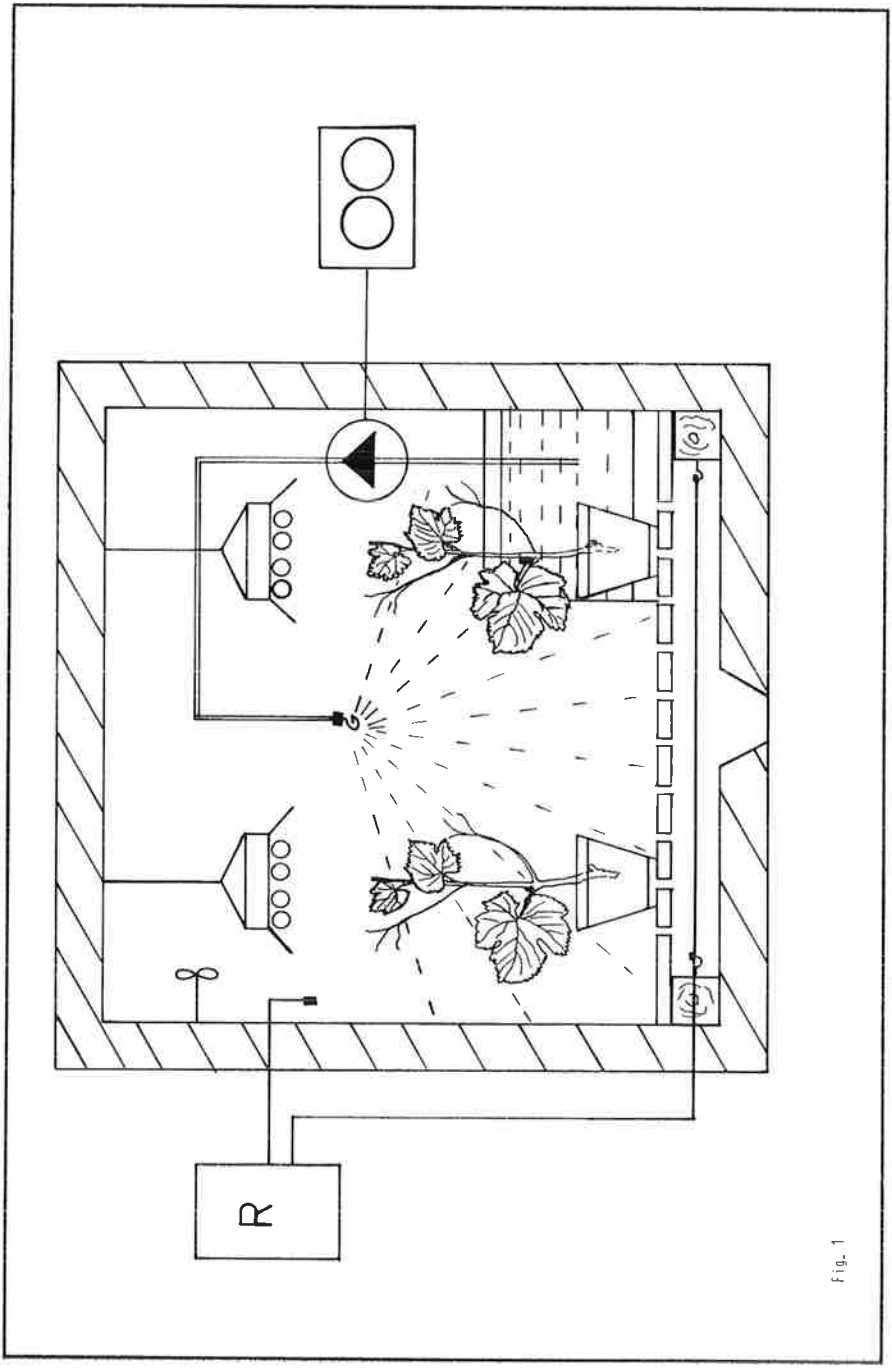

Fig. 1

PROCEDURE AND OPERATION

In preparation for heat treatments, vines were sprayed with a systemic fungicide. Some days after that, cuttings are taken, planted in vulcanic ashes, labeled and placed under mist into heat before they have had a chance to dry out.

Within three weeks under mist at 35-39°C new growth may start from the terminal or lateral buds. This growth is etiolated and useless for propagation. The cuttings begin to send out roots simultaneously. Most of the older leaves remain intact. Without leaves cuttings do not root.

Algae growth often develops a green coating on the leaves and around installations. This coating is not particularly harmful to the cuttings.

It may be that subjecting leaves to artificial rains will remove nutrients from the leaves. Nutrients added to the mist water in very low amounts are helpful.

Disease problems under mist conditions at high temperatures have not been serious. Probably the frequent washing of the leaves by the aerated water removes spores before they are able to germinate. Sometimes, the basal internode of the cuttings may be attacked by thermophylic fungi. Then rooting fails.

After the treatment at 35-39°C under mist conditions the cuttings growing in the clay pots are transferred to the above mentioned second type of growing chamber at 30°C for one week, and after that they are transferred back to the first position in order to continue the high temperature treatment. When growing under mist at 30°C recovery of the cuttings occurred.

RESULTS

The first experiments with NEPO-virus inactivation by treating greenhouse cuttings under mist were undertaken during May-July 1970 without continuation of the high temperature treatment after the recovery time. FS-4 vines infected with fanleaf or/and raspberry-ringspot viruses were heated at 35-39°C for three weeks and then transferred to the recovery-chamber. The plants were then removed and placed in the greenhouse. About 80% of the plants survived and sent out new growth. In November 1970 the plants had developed sufficient mature shoot tips for successful mist pro-

pagation. So far, in all plants propagated from tips no virus symptoms are to be seen. However, by early 1971 about 32% of the treated plants showed virus symptoms proving that fanleaf- and raspberry-ringspot-viruses in green grape cuttings could survive continuous exposure at 35-39°C under mist for three weeks, and that virus inactivation in entire plants was not very promising if there is no possibility to extent the time of treatment.

According to the above mentioned review, plant survival is increased by intermittent application of heat. This fact can be considered on their transference to 30°C for one week. After that time heat treatment can be continued for 30 days or more depending on varietal tolerance to heat and mist conditions.

Four weeks treated FS-4-indicators have been held in greenhouse for two years with frequent observations. All self-indexing plants have remained virus-free.

In experiments with vines of different varieties known to be infected by fanleaf, arabis mosaic, raspberry ringspot and tomato black ring viruses, similar results have been obtained.

For indexing fanleaf and or raspberry ringspot viruses, mature wood of treated plants and of plants propagated from the new growth was used for inoculating FS-4 indicator vines by bottle grafts.

To confirm the successful elimination of the other viruses, sap inoculations to *Chenopodium* indicators were carried out. Crude sap was never used. Mechanical transmissions have been repeated in time. Generally, all vines coming from heat treatment and all inoculated indicators are held in the greenhouse under mild climatical conditions.

Further experiments are under way. In these, the main question, whether I am under the necessity of doing virus tests after treatment generally or not, may be answered.

SUMMARY

Our experiments are showing that the probable destruction of essential chemical activities caused by high temperatures may be more effective in young green grape tissues than in woody ones. Heat treatment of green grape shoots is possible under mist. Large cuttings with considerable leaf

area can be treated, permitting the production of large-size NEPO-virus-free plants in a very short time, because there is no need in establishing mother plants in containers and the production of virusfree plants may start with green shoots from fruiting vines.

RIASSUNTO

I nostri esperimenti indicano che la probabile distruzione di attività chimiche essenziali determinata dalle alte temperature può essere più efficace nei giovani tessuti verdi di vite che non in quelli lignificati. Il trattamento termico di germogli verdi di vite è possibile in presenza di « mist ». In tal modo si possono trattare talee di grandi dimensioni, provviste di notevoli superfici fogliari, ottenendo la produzione di viti esenti dai virus NEPO in brevissimo tempo; infatti non occorre sistemare le piante madri delle talee in contenitori, bensì si possono ottenere piante virus-esenti partendo direttamentt dalle viti in produzione.

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I.N.I.A. - AVDA. PUERTA DE HIERRO s/n, MADRID-3 - SPAIN

A NEW AND ACCURATE WAY OF HEAT THERAPY OF PLANTS GROWN « IN VITRO » APPLIED TO THE SANITARY SELECTION OF SPANISH GRAPEVINE VARIETIES

A. PEÑA-IGLESIAS and PILAR AYUSO

The identification of the Grapevine Fanleaf virus (PEÑA-IGLESIAS A. and AYUSO, 1971) in different Spanish regions (Almería, Jerez, Cataluña, Valladolid, Rioja, Montilla, etc.) has motivated for the Laboratory of Plant Virology (INIA) in collaboration with the National Department of Viticulture (INIA) to undertake a sanitary selection of the major Spanish varieties.

We have started with the following varieties:

— Moscatel de Málaga, Palomino y Pedro Ximenez . . . . . de Andalucía Occidental
— Macabeo, Parellada, Sumoll, Xarelo blanco y Xarelo rosado . . . . de Cataluña
— Airen y Bobal . . . . . de la Región Centro
— Garnacha, Garnacha blanca, Graciano, Malvasía, Mazuela, Tempranillo y Viura . . . . . de la Rioja
— Aledo y Ohanes . . . . . del Sureste

In this selection we chose the clones for each variety presenting at sight a better aspect among the various vines of the same variety owned by the National Department of Viticulture.

The previous indexing is under way on the following indicators:

Vitis Rupestris St. George, LN-33, Bacco 22-A, Mission and Cabernet Sauvignon.

At the same time a mechanical transmission to herbaceous test plants (*Chenopodium quinoa*) and eventually serology has been effected.

This indexing would enable the election of the most suitable indicators under our climatic conditions.

For the regeneration of the selected clones we followed the method of culture « in vitro » at 20°C (Galzy, 1964) of cuttings of 2-3 cm length on a nutrient agar medium. After sprouting and rooting and following new transplantations to new tubes, when the explants reach 8 cm length (7 to 9 leaves) the tubes are subject to one week's exposition at 33°C and after this period temperature is increased to 37°C during 70 days (Mur, Valat and Branas, 1972).

The treatment of the explants in the tubes is undertaken in a plastic container with heated distilled water at 37°C by a laboratory thermostat. This container has a hot water supply from other one through a float regulation. The water thermotherapy container is located in a room tissue culture under a light of 2000 lux (Gro. lux).

Until the acquisition or construction of a definitive air heat chamber we have used this new system bearing in mind its economical, simplicity and higher temperature precision; (for the 3 months treatment the exact 37°C was maintained which is more accurate than at the standard heat chambers). The exact temperature maintenance of this system suggests that it could be useful in the future.

Several varieties have been heat treated and the comparison of treated and untreated clones had other interests besides the objective to obtain healthy plants from the grapevine varieties above mentioned.

We have undertaken the regeneration of the grapevine of Spanish varieties and we hope to contribute with them, when

healty, to the I.C.V.G. The sanitary selection of grapevine Spanish varieties will be a novelty not a reiteration (although it was interesting) to the exchange list of indexed plant material.

RIASSUNTO

Un nuovo ed accurato metodo di termoterapia di piante allevate « in vitro », applicato alla selezione sanitaria di varietà spagnole di vite

Cloni di vite di varietà spagnole sono stati selezionati per via morfologica e poi sottoposti a termoterapia. Questa è effettuata deponendo piccole talee apicali (2-3 cm) in tubi contenenti un substrato nutritivo agarizzato e mantenendo inizialmente la temperatura a 20°C. Dopo che le talee hanno radicato e raggiunto uno sviluppo di circa 8 cm, la temperatura è portata a 33°C per una settimana e quindi a 37°C per 70 giorni. Il contenitore dei tubi, in materia plastica, è tenuto in una vasca contenente acqua termostatata e provvista di illuminazione a 2.000 Lux. Gli autori ritengono che il metodo da essi sperimentato dia risultati soddisfacenti grazie soprattutto alla possibilità di ottenere temperature precise ed uniformi.

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BUNDESFORSCHUNGSANSTALT FÜR REBENZÜCHTUNG GEILWEILERHOF,
SIEBELDINGEN (DEUTSCHLAND)

ERFAHRUNGEN MIT DER WÄRMETHERAPIE BEI REBVIROSEN

H. HAHN

Anlass zu den Versuchen war die Notwendigkeit, für Anbauversuche virusfreies Pflanzmaterial zur Verfügung zu haben. Für die eigenen Versuche wurden die Erfahrungen mitverwendet, die bisher von anderen Autoren gemacht werden konnten. Da für Anbauversuche grössere Mengen gesunden Materials benötigt werden, musste die Behandlungstechnik so modifiziert werden, dass möglichst schnell eine grössere Zahl von gesunden Pflanzen erzeugt werden konnte. Deshalb wurde die Behandlung in einem Kleingewächshaus durchgeführt, das innerhalb eines normalen Gewächshauses aufgebaut wurde. Die Zelle hatte eine Grundfläche von 2×6 m und eine Firsthöhe von etwa 2,5 m. Die Temperatur liess sich auf $38° \pm 1°$C konstant halten, ebenso die Luftfeuchtigkeit auf relativ 70%. Während der Behandlungszeit von 3 Monaten wurde die Zelle nicht geöffnet. Gegossen wurde von aussen. Etwa 600 Stupfer konnten gleichzeitig behandelt werden. Nach den bisherigen Erfahrungen liessen sich die nach der Behandlung entnommen Triebspitzen am besten in Nebelkammern zur Bewurzelung bringen. Da bei uns diese Möglichkeit nicht bestand, wurde versucht, auf verschiedenen Nährsubstraten in Petrischalen diese Bewurzelung durchzuführen. Die Luftfeuchtigkeit in den Petrischalen reichte immer aus. Auf Nähr-Agar entwickelte sich bei den meisten Trieb-

spitzen keine Wurzel, sondern nur üppiger Kallus. Auch Zusätze von Aminosäure und Wuchsstoffen besserten dieses Ergebnis nicht sehr. Dagegen konnte eine sehr befriedigende Ausbeute an bewurzelten Stupfern erzielt werden, wenn die Anzucht auf einem Torf-Sand-Gemisch erfolgte.

Nach etwa 8 Wochen konnten die Stupfer getopft werden. Die Behandlung mit 0,1%iger Chinosol- oder Benomyl-Lösung verhinderte den Befall mit Botrytis, ohne die Bewurzelung zu beeinträchtigen. Anstelle von Petrischalen können auch grössere Plastikschalen mit Glassbedeckung mit gleichem Erfolg verwendet werden. Bisher blieben in dieser Weise behandelte Reben über mehrere Jahre äusserlich gesund. Eine Nachprüfung durch die bekannten Testmethoden konnte noch nicht durchgeführt werden. Es ist bekannt, dass in den letzten Jahren bei einigen Sorten nach thermotherapeutischer Behandlung morphologische Veränderungen auftraten. Es sind deshalb Versuche im Gange, diese Befunde für die hier behandelten Sorten zu überprüfen und im Anbauversuch evtl. Leistungsänderungen zu untersuchen.

SUMMARY

Research on heat treatment of grapevine virus diseases

A description is given of a modified method of the thermotherapeutical treatment of new vine breeds. The propagation in petri dishes of shoot cuttings in a sterile sand/peat mixture was successful. The yield of rooted cuttings was equivalent to the normal yield in vine nurseries.

RIASSUNTO

Ricerche sulla termoterapia delle virosi della vite

Viene descritto un metodo modificato di trattamento termoterapeutico di nuove selezioni di vite. La propagazione di germogli apicali in piastre petri, in una miscela di sabbia e torba, si è rivelata molto proficua. La produzione di talee radicate ottenuta con questo metodo è risultata pari a quella ottenuta nei normali vivai.

EIDG. FORSCHUNGSANSTALT WÄDENSWIL (SCHWEIZ)

VIRUSKONTROLLE UND BODENDESINFEKTION

(ZUSAMMENFASSUNG)

W. KOBLET

In einem sehr alten, seit fast 1000 Jahren mit Reben bestockten Rebberg besteht eine starke Virusinfektion, die wir seit 1958 beobachten. Wir fanden zur Hauptsache Panaschure-Virosen und in kleinem Ausmasse auch die Blattrollkrankheit. 15-20 Jahre nach einer Neupflanzung mussten die Reben bereits wieder ersetzt werden. Im Jahre 1967 zeigte eine Parzelle mit 1200 Reben einen Virusbefall von mehr als 60% mit sehr kleinem Ertrag. Wir stellten zudem eine starke Bodeninfektion mit dem Virusvektor *Xiphinema vuittenezi* fest. Aus diesem Grunde wurde der Boden vor der Neupflanzung mit dem Nematizid Shell DD 500 l/ha und 1000 l/ha desinfiziert. Andere Parzellen erhielten Kehrichtkompost. Jede Versuchsgruppe umfasste rund 72 Reben. Im Jahre 1971, zwei Jahre nach der Behandlung, fanden wir Nematoden und Virussymptome nur in den unbehandelten und in den Kompost-Parzellen. Die mit Shell DD behandelten Gruppen wiesen weder *Xiphinema vuittenezi* noch Blattsymptome auf. Fünf Jahre nach der Bodendesinfektion (1973) fanden wir die folgende Anzahl Reben mit Virussymptomen je Wiederholung:

— Shell DD 1000 l/ha: 1, 0, 0, Shell 500 l/ha 2, 3. Kompost 3 m^3/a: 2, 3. Kontrolle: 3, 5, 9.

Die Beobachtungen werden weitergeführt .Im nächsten Jahr wird der Boden erneut auf Nematoden untersucht.

RÉSUMÉ

Observation de symptômes de virus et traitements du sol

Dans un très vieux vignoble où l'on a cultivé la vigne depuis près de 1000 ans il existe une forte infection de virus que nous observons depuis 1958. Nous y avons surtout trouvé la panachure et aussi un peu l'enroulement. Déjà 15-20 ans après une nouvelle plantation les vignes ont dû être remplacées.

En 1967 une parcelle de 1200 ceps était infectée à 60% et le rendement était fortement diminué. Nous avons aussi constaté une grave infection du sol par le vecteur de virus *Xiphinema vuittenezi*. Pour cette raison le sol a été partiellement désinfecté avant de replanter la vigne, avec le nématicide Shell DD, à raison de 500 l/ha et 1000 l/ha. D'autres lots ont reçu du compost de gadoue.

Chaque groupe d'essai contenait environ 72 ceps. En 1971, c'est-à-dire 2 ans après le traitement, nous avons trouvé des nématodes et des symptômes de virus dans les parcelles non traitées et dans les parcelles de compost. Les parcelles traitées au Shell DD étaient exemptes de *Xiphinema vuittenezi* et de symptômes au feuillage. En 1973, 5 ans après la désinfection du sol, nous avons trouvé les nombres respectifs suivants de souches avec des symptômes de virus dans chaque répétition :

— Shell DD, 1000 l/ha : 1, 0, 0 ; Shell DD, 500 l/ha : 2, 3 ; compost, 3 m^3/a : 2, 3 ; témoin : 3, 5, 9.

Le vignoble reste sous contrôle et le sol sera à nouveau analysé pour nématodes l'année prochaine.

SUMMARY

Observation of virus symptoms and soil treatments

In a very old vineyard, where vines have been cultivated for almost 1000 years, there is a heavy infestation of virus desease. Since 1958 we have observed this vineyard in order to follow the distribution of the virus infection. We mostly found the panachure virus and to lesser extent the leaf roll desease. 15-20 years after replanting, the vines were again infested with virus and they had to be replaced. In 1967 a parcel with about 1200 vines showed a virus infection of more than 60%, and the yield was very much reduced. We also found an infestation of the soil born virus vector *Xiphinema vuittenezi*.

Therefore, prior to replanting we decided to treat the soil with the nematicid Shell DD 500 l and 1000 l per hectar as well as with compost. The different plots had about 72 vines each. In 1971, two years after treatment, we found nematodes and virus symptoms only in the untreated and compost plots. The treated plots (Shell DD) were free of *Xiphinema vuittenezi* and leaf symptoms. In 1973, 5 years after soil desinfection, we found the following numbers of vines with virus symptoms in each repetition:

— Shell DD 1000 l/ha: 0, 0,1, Shell DD 500 l/ha: 2, 3. Compost 3 m^3/a: 2, 3. Control: 3, 5, 9.

This vineyard it still under observation, and there will be a new soil analysis for nematodes next year.

SELEZIONE E CERTIFICAZIONE

(tavola rotonda)

Nella mattina di mercoledì 19 settembre si è svolta una discussione a tavola rotonda sui problemi concernenti la selezione e la certificazione del materiale da propagazione della vite con particolare riferimento agli aspetti sanitari. Nel corso della riunione vari colleghi hanno illustrato i metodi e i regolamenti applicati o in via di applicazione nei rispettivi paesi. Riteniamo utile darne qui un resoconto schematico. Per Francia e Sud Africa pubblichiamo i testi inviatici rispettivamente dal Dr. Rives e dai Dr. Burger, van Heerden e Engelbrecht; per gli altri paesi, dai quali non ci è giunto un testo scritto, pubblichiamo brevi riassunti preparati dal Prof. Refatti.

FRANCIA

En France la sélection de clones est officiellement confiée à des organismes dits de sélection (ou « établissements A ») qui sont actuellement les stations de l'I.N.R.A. (Colmar, Bordeaux, Montpellier) et l'Association Nationale pour l'Amélioration Technique de la Viticulture (A.N.T.A.V.) Domaine de l'Espiguette au Grau-du-Roi.

Les clones sélectionnés, après avoir obligatoirement subi des épreuves d'indexage vis-à-vis du court-noué, de l'enroulement et de la marbrure soit par sérologie soit par greffage, et des épreuves pour évaluer leurs performances quantitatives et *qualitatives* pour les variétés greffons sont agréés par la Section « Vigne » du Comité Technique Permanent de la Sélection (C.T.P.S.) organisme officiel groupant les représentants des professionnels et des administrations intéressés, pourvu qu'ils soient exempts des trois viroses pour les porte-greffes, des deux premières pour les variétés de greffons et que leur performances soient jugées suffisantes.

La multiplication du matériel est faite en deux étapes qui sont confiées à des établissements dits de prémultiplication (ou « établissements B »). Ceux-ci sont agréés par le Ministère de l'Agriculture après enquête menée par le C.T.P.S. et l'Institut des Vins de Consommation Courante (I.V.C.).

La prémultiplication est faite obligatoirement sur le principe des familles sanitaires en terrain vierge et désinfecté et placé sous le contrôle direct de l'établissement. Ces champs de prémultiplication fournissent le « matériel de base » qui sert ensuite à créer des champs de multiplication, qui doivent être constitué de matériel de base pour les deux partenaires, en terrain désinfecté.

Ces champs de multiplication produisent le « matériel certifié » qui est traité et vendu par les pépiniéristes.

Tout ceci répond aux exigences des directives européennes des 9 Avril 1968 et 22 Mars 1971. Cependant l'application rigoureuse des règles très strictes de la multiplication généalogique en familles sanitaires jusqu'au stade de la prémultiplication, en permettant de retracer immédiatement l'origine de toute plante à ce stade constitue une garantie supplémentaire par rapport à un texte qui se contente de demander l'absence de *symptômes* (!) de virus dans les champs de multiplication.

La certification française, faite dans un souci de qualité et non exclusivement de rendement, offre donc des garanties supérieures aux exigences communautaires européennes. Il est dans nos intentions de la renforcer encore :

— en éliminant progressivement la marbrure qui peut être guérie par thermothérapie,

— en incluant parmi les viroses testées les deux nouvelles mosaiques décrites par VUITTENEZ et surtout le corky-bark indexé par LN 33 (en même temps que l'enroulement d'ailleurs).

(M. RIVES)

SUD AFRICA

Improved clonal rootstock and scion cultivars freed of all known virus or viruslike diseases will be maintained in limited numbers in a repository under direct State control. This nuclear material, which will be indexed at regular intervals on a standard range of woody and herbaceous indicators, will be released to a central organisation, operated by the Co-operatvie Wine Growers' Association (KWV), for multiplication on behalf of the State. From this source rootstock material will be distributed to registered nurserymen who will establish and maintain healthy rootstock mother blocks for use as grafting material. Grafted material will also be supplied to registered nurserymen from the KWV foundation block for the establishment of scion-wood mother blocks.

Although there is no reason at present to anticipate that viruses or viruslike organisms will spread into the foundation and mother blocks it has been decided, as a precautionary measure, to retest the health status of rootstock and scion-wood clonal selections including mother blocks within five years after they have been issued from the repository.

Registered nurseries participating in this scheme must meet required isolation regulations and are free from specified nematodes that are vectors of virus diseases and also free from certain soil-borne fungal and bacterial diseases. The production and marketing of State certified material will be controlled by a State Inspection Service who will carry out at least two visual inspections during the growing season and a final one during lifting. A random sample drawn from all participating nurserymen will be submitted annually for the detection of systemic or other diseases.

(J.D. BURGER, H.P. VAN HEERDEN e D.J. ENGELBRECHT)

AUSTRALIA

E' stata esposta la situazione attuale e quella a cui si tende di arrivare nel prossimo futuro.

A) *Organizzazione attuale*:

1. *Selezione* di materiale locale o di nuova introduzione, curata dalle Istituzioni di ricerca dello Stato.
2. *Moltiplicazione* del materiale selezionato a cura del Ministero dell'Agricoltura.
3. *Distribuzione* del materiale selezionato alle Cooperative di produttori che istituiscono i campi di piante madri.
4. *Cessione* del materiale ai vivaisti, che provvedono alla costituzione dei loro campi di piante madri e quindi alla produzione di piante bimembri per i viticoltori.

B) *Organizzazione a cui si tende arrivare*

1. *Introduction*, organizzazione che provvede ai saggi del materiale da prendere in considerazione.
2. *Depository National*, campo piante madri dello Stato ove far confluire il materiale di base risultato sano, che ogni 5 anni dovrà essere sottoposto a indexing.
3. *Government blocks*, campi di piante madri per la moltiplicazione del materiale di base.
4. *Grower blocks*, campi di piante madri delle organizzazioni degli agricoltori per la successiva moltiplicazione del materiale di base.
5. *Nurserymen*, campi di piante madri istituiti dai singoli vivaisti per la produzione di viti bimembri su scala commerciale.

(relatore: R.H. TAYLOR; riassunto: E. REFATTI)

CANADA

Molto del materiale di propagazione della vite viene importato dall'estero. Esso viene raccolto nell'isola di Vancouver e sottoposto a indexing. Si scarta tutto quanto non risulti esente da virus. Finora non è stata creata una organizzazione ufficiale per la produzione di materiale certificato. Per la moltiplicazione del materiale esente da virus (cvv. commerciali e portinnesti) si procede secondo lo schema che segue:

1. *Nuclear block*

Viene istituito presso le Stazioni Sperimentali ed è costituito da 2-3 piante di ciascun clone, allevate in vaso, in serra. Tutte le piante vengono sottoposte a saggi periodici su indicatrici.

2. *Foundation block*

È un campo di piante madri, costituito da 10-15 viti per ciascun clone selezionato, istituito presso le Stazioni Sperimentali, in terreno mai

coltivato a vite. Tutte le piante vengono sottoposte periodicamente all'indexing.

3. *Nurseryman coop foundation block*

È il campo di piante madri istituito dalle cooperative di vivaisti, con una superficie di 4.000-6.000 mq. I saggi su indicatrici vengono effettuati « per campione » su poche piante di ciascuna cv. o portinnesto.

4. *Nursery foundation block*

Ogni singolo vivaista istituisce il proprio campo di piante madri, con una superficie dell'ordine di 8.000 mq, da cui prelevare il materiale necessario per la produzione di piante bimembri. In esso vengono fatti controlli a vista da parte di ispettori specializzati. Per ora, si considera che il vigneto piante madri possa fornire materiale certificabile per 8 anni, dopo di che il vivaista dovrà ricostituire un nuovo campo piante madri ed usare eventualmente il vecchio vigneto per la produzione di materiale comune.

(relatore: H. Dias; riassunto: E. Refatti)

GERMANIA OCC.

Da 30-40 anni si fa la selezione a vista, per la conservazione del materiale con buone caratteristiche varietali e produttive, scartando le piante con sintomi evidenti o sospetti di virosi.

In futuro si conta di applicare le norme previste dal regolamento della CEE, nel quale è stabilito che le piante destinate alla produzione di materiale di base devono essere esenti da virus, con speciale riguardo all'arricciamento ed all'accartocciamento fogliare. Per la produzione di materiale certificato e standard, il regolamento prevede, invece, che vengano escluse le piante che presentano sintomi visibili di virosi.

1. *Produzione di materiale di base*

Si fa la selezione mediante indexing (periodo di osservazione delle piante indicatrici per l'accartocciamento fogliare 3 anni), test sierologici e saggi su piante test erbacee (*).

Ciascun clone esente da virus e con buone caratteristiche varietali viene moltiplicato per talea e quindi sottoposto nuovamente all'indexing. Se risulterà sano si conserveranno poche piante in serra, mentre le altre verranno moltiplicate in campo, in terreno sottoposto a fumigazione.

2. *Produzione di materiale certificato*

Si innesta il materiale di base su portinnesti esenti da virus onde ottenere le piante madri, che poi serviranno per la produzione delle piante bimembri.

(relatore: G. Stellmach; riassunto: E. Refatti)

(*) Le capacità di lavoro delle Stazioni sperimentali sono attualmente modeste, ma si conta di poter arrivare a un notevole potenziamento delle stesse nei prossimi 5-10 anni.

U.S.A. - CALIFORNIA

Tutto il materiale usato per la riproduzione deve essere sottoposto a quarantena. È proibita l'importazione dall'Europa.

L'organizzazione per la produzione di materiale certificato è la seguente:

1. *Foundation block*

È costituito presso l'Università di California (U.C.) con materiale (portinnesti e cvv commerciali) esente da virus, saggiato sulle varie indicatrici e sottoposto a due ispezioni annuali.

2a. *Field mother block*

È costituito nei campi di U.C., immettendovi per la moltiplicazione il materiale del Foundation block, che viene sottoposto a controlli da parte di specialisti dell'Extention service.

2b. *Greenhouse mother block*

Serve anch'esso per la moltiplicazione del materiale del Foundation block. È costituito nelle serre di U.C., con talee poste in vaso e che vi permangono per 12 mesi. Le piante vengono sottoposte ai controlli da parte degli Extention men.

3a. *Increase in field*

Il materiale ottenuto nel Field mother block viene passato a vivaisti specializzati e selezionati (facendolo pagare notevolmente in modo da coprire le spese sostenute per produrlo), che provvedono a moltiplicarlo. Esso viene sottoposto a controlli da parte degli Extention men.

3b. *Increase in greenhouse*

Le piante in vaso del Greenhouse mother block vengono passate a organizzazioni vivaistiche specializzate ed autorizzate, che provvedono alla moltiplicazione con il sistema del « mist ».

4. *Nursery mother blocks*

Il materiale ottenuto nella fase di moltiplicazione, di cui in 3a) e 3b) viene passato ai vivaisti, che istituiscono i loro campi di piante madri, dalle quali preleveranno le marze ed i portinnesti da impiegare nella produzione di viti bimembri per gli agricoltori.

(relatore: A.C. Goheen; riassunto: E. Refatti)

RIUNIONE ORGANIZZATIVA

(Administrative meeting)

La sera del 17 settembre si è riunito, in via preliminare, il Comitato Internazionale dell' I.C.V.G. Successivamente, nella mattina del giorno 19, si è tenuta la riunione generale con lo scopo di discutere i futuri problemi organizzativi. Delle decisioni prese si dà qui un breve resoconto preparato in lingua inglese dal Dr. R. Bovey, segretario dell'I.C.V.G.

1. *Next meeting of the ICVG*

Three possibilities were presentend: Spain, Yugoslavia and Canada. After a short discussion, they were put to vote. Spain was chosen, Yugoslavia and Canada came second and third respectively. It was decided to hold this meeting in 1976 and to try to arrange it just after or before the next International Symposium on Fruit Tree Virus Diseases, which is to take place the same year at Heidelberg, Germany, so that colleagues from other continents interested in both meeting can attend them in one trip. This follows a wish expressed also by several members of the Symposium on Fruit Tree Virus Diseases.

2. *List of virus-tested grapevine material*

A new list of virus-tested grapevine material available in 10 Institutes has been prepared and distributed at Salice Terme. More copies are available from dr. R. Bovey.

3. *Bibliography*

The bibliography on grapevine viruses and virus diseases, also including mycoplasma-like diseases, has been prepared by Dr. Caudwell for the period 1965-1970 and published in Vitis 11, 302-324, 1972, with a text by W.B. Hewitt, R. Bovey and A. Caudwell. This paper should be quoted as follows: Caudwell, A., Hewitt, W.B., Bovey, R., 1972. Les viroses de la vigne. Bibliographie de 1965-1970, Vitis, 11, 303-324.

4. *List of antisera*

Dr. Martelli has tried to set up a list of antisera available for exchange between members of ICVG, but his questionnaire met very limited success. He will try again.

5. *Handbook on symptoms of virus, mycoplasma and deficiency diseases of the grapevine*

A swiss publisher is possibly interested in publishing this handbook if he can obtain enough certinty about the possibility of sale. This handbook would contain a short introducion on grapevine virus, mycoplasma, and deficiency diseases in French and English, and colour or

black and white photographs of the symptoms, with legends in French, English, German, Italian and Spanish. It as also been suggested to prepare sets of slides on te symptoms of these diseases. Contacts will be made with EPPO on this matter.

6. *International Society of Plant Pathology*

Dr. Martelli, who represented our group at the 2nd International Meeting of Plant Pathology at Minneapolis (USA) in September 1973 reported on the proposal of the International Society of Plant Pathology (ISPP) that ICVG should join this Society as an associated or affiliated organization. The Committee of ICVG has decided to accept this proposal, and has made contact with the Committee of the ISPP on this matter.

Partecipanti al 5° Convegno I.C.V.G.

ELENCO DEI PARTECIPANTI

AMICI Adriana	— Istituto di Patologia Vegetale, Università di Milano (Italia).
BALDACCI E.	— Istituto di Patologia Vegetale, Università di Milano (Italia).
BELLI G.	— Istituto di Patologia Vegetale, Università di Milano (Italia).
BETTO E.	— Istituto di Patologia Vegetale, Università di Milano (Italia).
BARNA J.	— Versuchsanstalt fuer Obst - u. Weinbau, Klosterneuburg (Austria).
BASSO M.	— Istituto di Coltivazioni Arboree, Università di Pisa (Italia).
BERCKS R.	— BBA - Abteilung für pflanzliche Virusforschung, Braunschweig (Germania occ.).
BESTAGNO G.	— Osservatorio Malattie delle Piante, San Remo (Italia).
BEUKMAN E.	— Cooperative Winegrowers Association (KWV), Paarl (Sud Africa).
BLEVE Teresa	— Laboratorio Nematologia Agraria (C.N.R.), Bari (Italia).
BOVEY R.	— Station fédérale de recherches agronomiques, Nyon (Svizzera).
BRUGGER J.J.	— Station fédérale de recherches agronomiques, Nyon (Svizzera).
BURGER J.	— Research Institute for Oenology and Viticulture, Stellenbosch (Sud Africa).
CANOVA A.	— Istituto di Patologia Vegetale, Università di Bologna (Italia).
CASINI E.	— Istituto di Coltivazioni Arboree, Università di Firenze (Italia).
CATARA A.	— Istituto di Patologia Vegetale, Università di Catania (Italia).
CAUDWELL A.	— Station de Physiopathologie Végérale (I.N.R.A.), Dijon (Francia).
COIRO M.	— Laboratorio Nematologia Agraria (C.N.R.), Bari (Italia).
CONTI G.G.	— Istituto di Patologia Vegetale, Università di Milano (Italia).
CONTI M.	— Laboratorio di Fitovirologia Applicata (C.N.R.), Torino (Italia).
COSOLO GIUSSANI Alessandra	— Centro Regionale per la Sperimentazione Agraria, Udine (Italia).
DE FANTI L.	— Osservatorio Malattie delle Piante, Verona (Italia).
DEL ZAN F.	— Centro Regionale per la Sperimentazione Agraria, Udine (Italia).
DIAS H.	— Canada Agriculture Research Station, Vineland Station, Ontario (Canada).
DI LORENZO B.	— Istituto Tecnico Agrario, Ascoli Piceno (Italia).

DI MATTEO C. — Istituto Tecnico Agrario, Ascoli Piceno (Italia).

DIMITRIJEVIC B. — Institute for Plant Protection, Beograd (Jugoslavia).

DOAZAN J.P. — Station de Recherches de Viticulture, Pont de la Maye (Francia).

EGGER E. — Istituto Sperimentale per la Viticoltura, Conegliano (Italia).

ENGELBRECHT D.J. — Plant Protection Research Institute, Stellenbosch (Sud Africa).

FARINA Gandolfina — Istituto di Patologia Vegetale, Università di Milano (Italia).

FOGLIANI G. — Istituto di Patologia Vegetale, Facoltà Agraria, Piacenza (Italia).

FORTUSINI A. — Istituto di Patologia Vegetale, Università di Milano (Italia).

GALBIATI C. — Istituto di Patologia Vegetale, Università di Milano (Italia).

GIUNCHEDI L. — Istituto di Patologia Vegetale, Università di Bologna (Italia).

GOHEEN A.C. — Plant Pathology Department, University of California, Davis (U.S.A.).

GOMEC Belgin — F.A.O. Agency, Izmir (Turchia).

HAHN H. — Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, Siebeldingen (Germania occ.).

HARTMAIR V. — Lehranst. Obst - u. Weinbau, Klosterneuburg (Austria).

HEVIN M. — Station de Recherches de Viticulture, Point de la Maye (Francia).

HEWITT W.B. — Agricultural Research and Extension Center, University of California, Parlier, Calif. (U.S.A.).

KOBLET W. — Eidg. Forschungsanstalt, Wädenswil (Svizzera).

KUSZALA J. — Station de Pathologie Végétale, Colmar (Francia).

LAMBERTI F. — Laboratorio Nematologia Agraria (C.N.R.), Bari (Italia).

LEGIN R. — Station de Pathologie Végétale, Colmar (Francia).

LOCCI R. — Istituto di Patologia Vegetale, Università di Milano (Italia).

LODI P.L. — Fondazione Agr. « C. Gallini », Voghera (Italia).

LORETI F. — Istituto di Coltivazioni Arboree, Università di Pisa (Italia).

MARTELLI G. — Istituto di Patologia Vegetale, Università di Bari (Italia).

MENDONCA Amarillis — Estaçao Agronomica Nacional, Oeiras (Portogallo).

MORI P. — Osservatorio per le Malattie delle Piante, Verona (Italia).

MOSCONI R. — Fondazione Agr. « C. Gallini », Voghera (Italia).

NIENHAUS F. — Institut für Pflanzenkrankheiten, Bonn (Germania occ.).

OSLER R. — Istituto di Patologia Vegetale, Università di Milano (Italia).

PEÑA A. — Estasion de fitopatologia Agricola, I.N.I.A., Madrid (Spagna).

PEÑA - AYUSO Pilar — Estacion de fitopatologia agricola, I.N.I.A., Madrid (Spagna).

PICCO D. — Osservatorio Malattie delle Piante, Pavia (Italia).

PIERI G. — Istituto Sperimentale per la Viticoltura, Conegliano (Italia).

POP J. — Institutul central de carcetari agricole, Bucarest (Romania).

PROTA U. — Istituto di Patologia Vegetale, Università di Sassari (Italia).

PUTZ C. — Station de Pathologie Vegetale, Colmar (Francia).

QUACQUARELLI A. — Istituto di Patologia Vegetale, Università di Bari (Italia).

REFATTI E. — Istituto di Patologia Vegetale, Università di Catania (Italia).

RIVES M. — Station de Recherches de Viticulture, Pont de la Maye (Francia).

ROCA F. — Laboratorio Nematologia Agraria (C.N.R.), Bari (Italia).

ROSCIGLIONE B. — Istituto di Patologia Vegetale, Università di Palermo (Italia).

RÜDEL Maria — Landes - Lehr - und Forschungsanstalt für Wein-und Gartenbau, Neustadt/Weinstr. (Germania occ.).

RUI D. — Lungadige Panvinio 15, Verona (Italia).

RUSSO M. — Istituto di Patologia Vegetale, Università di Bari (Italia).

SARIC Ana — Poljoprivredni Fakultet, Zagreb (Jugoslavia).

SCARAMUZZI F. — Istituto di Coltivazioni Arboree, Università di Firenze (Italia).

SCARAMUZZI G. — Istituto di Patologia Vegetale, Università di Pisa (Italia).

SCHOEFFLING H. — Versuchsanstalt fuer Weinbau, Trier (Germania occ.).

SCHVESTER D. — Station de Recherches Forestieres, Avignon (Francia).

SERINI V. — Soc. Monteshell, Milano (Italia).

STELLMACH G. — BBA - Institut für Rebenkrankheiten, Bernkastel (Germania occ.).

TANNE Edna — Volcani Center of Agricultural Research, Bet Dagan (Israele).

TAYLOR R.H. — Victoria Plant Institute, Burnley (Australia).

TODESCHINI E. — Vivai Cooperativi, Padergnone (TN) (Italia).

TRIOLO E. — Istituto di Patologia Vegetale, Università di Pisa (Italia).

UGOLINI A. — Osservatorio Malattie delle Piante, Torino (Italia).

VALAT C. — Domaine de l'Espiguette (A.N.T.A.V.), Le Grau-du-Roi (Francia).

VEGETTI G. — Istituto di Patologia Vegetale, Università di Milano (Italia).

VIDANO C. — Istituto di Apicoltura, Università di Torino (Italia).

VOYLAS C. — Istituto di Patologia Vegetale, Università di Bari (Italia).

VUITTENEZ A. — Station de Pathologie Végétale, Colmar (Francia).

WEISCHER A. — Biol. Bundesanstalt, Münster (Germania occ.).

ZANARDI D. — Osservatorio Malattie delle Piante, Cagliari (Italia).

ZELGER F. — Ispettorato Provinciale Agricoltura, Bolzano (Italia).

COMITATO ORGANIZZATORE

Prof. E. BALDACCI — (*Presidente del 5° Convegno*) - Direttore dell'Istituto di Patologia Vegetale, Università di Milano.

Prof. G. BELLI — (*Segretario del 5° Convegno*) - Istituto di Patologia Vegetale, Università di Milano.

Prof. W.B. HEWITT — (*Presidente dell'I.C.V.G.*) - Agric. Research Center, University of California, Parlier, Calif. (U.S.A.).

Prof. R. BOVEY — (*Segretario dell'I.C.V.G.*) - Station Fédérale de Recherches Agronomiques, Nyon (Svizzera).

Prof. P.L. LODI — Commissario della Fondazione Agr. Gallini, Voghera.

Prof. F. LORETI — Direttore dell'Istituto di Coltivazioni Arboree, Università di Pisa.

Prof. D. PICCO — Direttore dell'Osservatorio per le Malattie delle Piante di Pavia.

Prof. E. REFATTI — Direttore dell'Istituto di Patologia Vegetale, Università di Catania.

Prof. F. SCARAMUZZI — Direttore dell'Istituto di Coltivazioni Arboree, Università di Firenze.

Prof. G. SCARAMUZZI — Direttore dell'Istituto di Patologia Vegetale, Università di Pisa.