PSOROSIS

Psorosis - A Review

C. N. Roistacher

ABSTRACT. Psorosis, as originally described, is a disease of citrus which induces typical bark scaling lesions in the trunks and limbs of sweet orange, mandarin and grapefruit, and occasionally ringspot symptoms on leaves and fruit. Wood staining often accompanies bark scaling in infected branches and trunks. Psorosis-infected budwood will induce a variety of symptoms on leaves of inoculated indicator seedlings of sweet orange, grapefruit or mandarin. These include shock, flecking, various patterns, blotching, blisters, ringspots, and chlorosis. Diseases which should not be included in the psorosis complex are concave gum, impietratura, cristacortis, blind pocket, crinkly leaf, infectious variegation, Dweet mottle, psorosis-like-pathogens (from Spain), the satsuma dwarf complex of viruses and the seed-transmitted psorosis-like disease reported from Florida. These diseases can be separated from psorosis by symptoms in field trees, by reaction on indicator plants and by cross protection tests using psorosis-B lesion inoculum as the challenge inoculum. Cross protection still remains the reliable standard for judging relationship to psorosis. Psorosis is spread primarily by man via infected propagative budwood. Natural spread has been shown but a vector has not been identified. Seed transmission has not been demonstrated. Recent studies implicate two different virus particles for psorosis; a unique flexuous two component virus containing a 48-kd capsid protein and a flexuous rod-shaped carlavirus-like particle containing a 29-kd protein (Levy & Gumpf, 43). Mechanical and graft transmissions were done by Garnsey and Timmer (37) from infected sweet orange to citron, then from citron to various herbaceous hosts and ultimately from herbaceous hosts back to sweet orange which later developed typical psorosis-B lesions.

This review suggests criteria for classifying the psorosis disease, reviews seedling and mechanical inoculation to indicator plants, gives the method for cross protection and methods for elimination of psorosis from propagative budwood. A detailed review is given of the association of ringspot to psorosis. The evidence presented and the consensus of many workers suggests that ringspot is a severe form of psorosis and should not be classified as a separate virus.

Psorosis is our oldest researched citrus virus disease. It was the first of the citrus diseases proven to be graft transmissible (29,30) and the first to be detected via graft-transmission to seedlings (74). The discovery that psorosis was transmissible led to the first eradication program (25) and the first certification program (31) for a citrus disease. At one time, psorosis was the most destructive disease of citrus. Today, because of natural spread of severe forms of the disease, psorosis is still a very serious and destructive disease in some countries.

There are several diseases reported with the same or different names in different countries which suggest they are related to psorosis since they induce varying leaf symptoms on trees or index plants. There is great biological variability reported for different psorosis isolates resulting in complications in symptomatology by mixed infections with other viruses. The etiology of this leaf-flecking group of viruses has not as yet been established. There is much confusion in the literature on just what is psorosis since very similar syndromes have been named as psorosis or ringspot depending on symptoms, the country of origin or the author. One objective of this review is to bring together recent studies on psorosis to clarify the various and divergent names now in use for diseases which have been classified as belonging to the psorosis group. Also an attempt is made to clarify the relationship of the citrus ringspot virus to psorosis.

The history of psorosis has been previously reviewed (69, 77, 78). Current information on symptomatology,

plus seed, pollen and possible vector transmission is reviewed. Current research on the identity of the pathogen(s) associated with the disease is presented. Methods for indexing, cross-protection and elimination of the pathogen from propagative budwood are given and the early programs for certification and eradication of psorosis are presented.

PSOROSIS DEFINED

Psorosis, as originally described by Swingle and Webber (1896) is a disease associated with typical bark scaling in trunks and limbs of sweet orange, mandarin and grapefruit. In addition to bark scaling, certain leaf symptoms were found associated with the disease and these symptoms were reproduced by graft transmission (29). Wallace (74) later showed that when buds taken from suspect trees were graft-inoculated to sweet orange indicator seedlings, shock and young leaf symptoms were produced and these were diagnostic for psorosis.

Two types of psorosis were proposed by Fawcett and Klotz (32): psorosis-A and psorosis-B. Both had similar bark lesion symptoms "*but in older, mature leaves the 'B' type may show larger chlorotic ring spots — symptoms on fruit are rare in the A type but in the B type are often large, discolored circular to semi-circular rings or grooves*". Both the A and the B types of psorosis were later shown by Wallace (75) to be related by cross protection. The A type protected against a challenge by the B type. Wallace proposed that all psorosis-A contained the psorosis-B component or strain, but internal cross protection delayed bark lesion expression. Wallace also proposed that the two strains or components (A and B) were systemic in all infected trees. He reported that "*prior to lesion formation, component A, possibly because of its more rapid increase and higher concentration, prevents component B from increasing in concentration to a point sufficient to offset the presence of A*". Wallace suggested that the concentration of the B component became dominant in the bark and overcame the protective influence of the A component which resulted in the development of the diagnostic bark lesions. This internal protection usually broke down after 12 to 16 yr or longer and bark lesions appeared. However, if bark lesion inoculum is taken from a field tree and inoculated into a non-infected sweet orange seedling tree, bark scaling can begin in less than five months (33). Presumably there was no protection by the "A" component which was not present in seedling trees, and the psorosis became rampant. Non-lesion psorosis (psorosis-A) could be identified by its ability to protect sweet orange seedlings against a challenge with the severe psorosis-B lesion inoculum. In addition to bark scaling symptoms, the presence of an internal wood staining in severely affected sweet orange limbs was also shown to be associated with the disease.

Classification and types of psorosis. There are a number of graft-transmissible diseases of citrus which have been called psorosis primarily because they induced leaf symptoms in inoculated test plants. Initially, possibly all diseases which produced flecking in leaves of sweet orange were put in the psorosis group (34). These were: concave gum, blind pocket, crinkly leaf and infectious variegation. Florida seed transmitted psorosis (8,15) and Monak psorosis in Australia (9) were defined as psorosis but were never challenged with psorosis-B and bark lesions were never observed. In their comprehensive review of psorosis, Timmer and Beñatena (69) stated that the viruses of crinkly leaf, infectious variegation, satsuma dwarf, citrus mosaic, navel infectious mottle, natsudaidai dwarf and citrus leaf rugose should *not* be included in the psorosis group of viruses since they had been purified and are viruses with spherical particles 26 to 32 nm diameter. The infectious variegation virus does not protect against a challenge from psorosis-B lesion inoculum (16). Concave gum, impietratura and cristacor-

tis also should not be grouped with psorosis (64). These diseases all produce oak leaf patterns in leaves of field trees as well as indicator plants and they rarely induce shock symptoms in indicator plants. The concave gum pathogen will not protect against a challenge from psorosis-B lesion inoculum (57). They do not produce scaly bark but induce other trunk or fruit symptoms distinct from that of psorosis. Also, da Graça *et al.* (18,19) showed that isolates of concave gum, impietratura, and cristacortis did not contain a 48-kd protein commonly associated to psorosis and ringspot isolates. Blind pocket is probably a variant of concave gum and until investigated further should not be included in the psorosis group. The Dweet mottle virus in sweet orange did not protect against a challenge from either psorosis-B lesion inoculum or concave gum and should be considered as a distinct and separate disease (58). The psorosis-like-pathogen (PLP) reported from Spain appears different from psorosis and does not protect against a challenge from psorosis-B (3,46). The PLP from Spain might be a concave gum, impietratura or cristacortis without obvious symptoms (46). A transmissible leaf variegation and fruit spotting disease reported by Planes and Martí (50) in Spain is probably not related to psorosis or ringspot and cross protection was not done. A number of new leaf pattern diseases have recently been reported, most or all of which are probably not related to psorosis. These include: The ring pattern disease of sweet orange reported from Iran (20, 27), citrus measles reported from Florida and Brazil (42), yellow vein clearing of lemon in Pakistan (14), the new graft-transmissible disease of pummelo in India with particles resembling a rhabdovirus (2) and citrus mosaic in India (1).

Diseases which can be considered as belonging to the psorosis group are: psorosis-A, psorosis-B, ringspot, the necrotic strains of ringspot (47, 68), naturally spread psorosis in Argentina and Uruguay and possibly the eruptive psorosis on fruit reported from Argentina (54).

A suggested criterion for judging a disease as belonging to the psorosis group might be the presence of most or all of the following symptoms or conditions: 1) presence of classical scaly bark lesions, primarily in trunks and limbs of sweet orange, grapefruit, and mandarins and occasionally but rarely in lemon. *However, it is important to realize that the psorosis virus may be present in symptomless trees!* 2) finding of classical wood staining in the cut limbs of mature branches or trunks of sweet orange which show scaly bark. 3) shock symptoms induced in sweet orange, mandarin, citron or lemon indicator plants grown under cool greenhouse conditions (66). 4) protection of an inoculated sweet orange seedling after a challenge with psorosis-B lesion inoculum. 5) ring patterns and mature leaf symptoms found on fruit and leaves of field trees. 6) raised blisters on leaves, stems and thorns of inoculated sweet orange plants or 7) raised blisters found on stems, leaves and thorns of field trees from shoots usually found near bark lesions. Bark lesion inoculum from mature trees grafted to sweet orange seedlings will induce these psorosis-B type lesion symptoms on stems, leaves and thorns of the sweet orange. 8) Mechanical transmission from infected citron to *Chenopodium quinoa* and subsequent observation of the top and bottom components containing the 48-kd protein (see purification).

The test of cross protection, originally proposed by Wallace (75) using psorosis-B lesion inoculum as the challenge inoculum is still the most reliable diagnostic tool for determining if a given syndrome is related to psorosis-A. However, one must always be aware that mixtures of viruses may be present. This was probably responsible for the initial inclusion of the viruses of crinkly leaf, infectious variegation and concave gum in the psorosis complex (34, 77). If protection against psorosis-B challenge inoculum occurs, it is reasonable to assume that the infected plant

or tree contains the psorosis-A pathogen, but it could also contain other pathogens which are not psorosis but can cause leaf symptoms which are generally more striking.

DETECTION OF PSOROSIS*

Field symptoms

Bark scaling. Psorosis-A can be diagnosed in the field if the symptoms of bark scaling and wood staining of stems are observed. Bark scaling alone, though usually diagnostic, should not be totally relied upon for identification. Bark lesions which are not psorosis such as Rio Grande gummosis of grapefruit, Phytophthora in sweet orange, shell bark of lemon or leprosis of sweet orange can usually be differentiated from psorosis by the character of the lesions and by indexing from budwood of suspect trees to sweet orange indicator seedlings.

The major susceptible varieties which show psorosis bark scaling are sweet orange, mandarin and grapefruit. The sour orange, sour lemon, pummelo and rough lemon usually show no external bark symptoms. It is important to remember that many varieties of citrus will carry the psorosis virus *without* showing bark scaling or leaf symptoms in the field tree and are symptomless carriers. The presence or absence of the virus can only be verified by indexing.

Leaf and fruit symptoms. Psorosis-B may show varying leaf and fruit symptoms including ringspot leaf patterns. In California, South Africa, and perhaps elsewhere, field trees with psorosis bark lesions may not show leaf patterns in the young growth flushes (26, 64), except in mixed infections. Therefore, looking for leaf symptoms is not recommended for diagnosis of psorosis-A. In contrast, the oak-leaf pattern associated with concave gum, impietratura or cristacortis diseases are abundantly present in field trees in cooler regions of the world, especially in the Mediterranean region and usually in the spring and fall flush of growth. The appearance of the oak-leaf pattern in leaves of field trees should *not* be mistaken for psorosis-A, or called psorosis.

Seedling indexing

The first use of citrus seedlings to detect a graft-transmissible pathogen in citrus was done by Wallace (74) for detection of psorosis-A. This seedling index reduced the time required for indexing from an average of approximately 11 yr for development of bark lesions in field trees to about 6 weeks for symptom development in the young leaves of sweet orange indicator seedlings. This revolutionary development pioneered the rapid detection, not only of psorosis, but of other graft-transmissible citrus pathogens by indexing via graft-transmission to greenhouse-grown plants and opened the door to certification.

Currently the primary means of detecting psorosis-A is by graft transmission to seedlings of sweet orange. Many psorosis isolates are difficult to transmit mechanically and primary identification must be by seedling index with verification by cross protection. Citron, Dweet tangor, certain mandarins and the sour lemon are also excellent indicators for psorosis-A. The sweet orange seedling is the preferred indicator, and Pineapple, Madam Vinous and Olivelands sweet orange have been found to be superior indicator varieties whereas Koethen, Mediterranean or Diller sweet oranges should not be used (56). The Dweet tangor is an especially sensitive indicator for psorosis as well as other diseases which induce leaf patterns like the oak-leaf pattern inducing diseases of concave gum, impietratura and cristacortis, or leaf patterns associated with infectious variegation, and the Dweet mottle virus.

The temperature during the first 4 weeks after inoculation is critical for

*da Graça *et al.* (18, 19) showed that isolates of concave gum, impietratura, and cristacortis did not contain a 48-kd protein commonly associated to psorosis and ringspot isolates.

symptom expression. Cool temperatures favor the appearance of shock reactions in the young emerging shoots whereas warm temperatures may inhibit shock reactions and mask leaf symptoms. Psorosis-A symptoms are best expressed at relatively cool temperatures of 24 to 27 C maximum day and 18 to 21 C minimum night. Shock and leaf pattern symptoms *may not appear* if temperatures are too warm. The critical period for development of shock and young leaf symptoms is during the first and second flush of growth after the inoculated plants are cut back.

Recently, Guirado (39) induced psorosis symptoms at warm greenhouse temperatures (average 30 C) by growing plants and inoculating them at the warm temperatures and then transferring the inoculated plants to growth chambers at 15 C for 3-6 days (16 hr of light) when 3-4 leaves were just emerging. Plants were then transferred back to the warm temperature greenhouse to continue their growth flush. Shock and excellent young leaf symptoms developed even under the warm greenhouse conditions. Navas-Castillo and Moreno (48) found that the effect of the incubation temperature was dependent on the host-isolate combination. In some of these combinations symptoms at warm temperatures were more intense then at cool temperatures, whereas in other combinations the contrary occurred.

Cross protection

To determine if the virus *is* psorosis-A, the inoculated sweet orange seedling should be challenge-inoculated with psorosis-B lesion inoculum and observed for evidence of cross protection. The source of psorosis-B as inoculum for use in cross protection tests is obtained by grafting lesion inoculum (taken from bark lesions of the trunk or limbs of a field tree) to a sweet orange seedling. Under proper temperature conditions, blister-like lesions will form on the stems in 6 to 8 weeks, and later develop on the leaves. Lesions usually form near the initial challenge inoculation site. If lesions develop on the challenged test plants, then psorosis-A is *not* indicated. Conversely, if lesions do *not* develop on the preinoculated but challenged test plants, but develop abundantly on the non-preinoculated but challenged controls, then the plant in question is most probably infected by psorosis-A or a mixture of psorosis-A and other leaf-flecking viruses. Young plants showing blister lesions can be used as challenge inoculum and held as source plants in a cool greenhouse. Tissue selected for challenge inoculum should preferably show the bark-blister symptoms.

Mechanical transmission from citrus to citrus and from citrus to herbaceous hosts.

The method recommended for mechanical transmission is that of Garnsey and Timmer (37). Symptomatic young leaf tissue is ground in cold buffer TME (0.05M Tris buffer pH 8.0 plus 0.5% 2-mercaptoethanol) using pre-chilled mortars and pestles and applied immediately with cotton swabs to leaves pre-dusted with 500-mesh carborundum. Temperatures after inoculation should be at 21 to 27 C. Symptoms on *C. quinoa* will appear in 4 to 6 days as chlorotic local lesions. Garnsey and Timmer (38) succeeded in mechanically transmitting Florida, Texas and California (psorosis) ringspot isolates, plus three California psorosis-B isolates from citrus to *C. quinoa*. They could not mechanically transmit any isolate showing symptoms in *C. quinoa* back to citrus, but could transmit ringspot isolates from *C. quinoa* to *Gomphrens globosa* and then back to citrus.

Mechanical transmission from citrus to citrus is done best by knife or razor slash into the stem. Citron is both an excellent host and receptor plant. Again, it is important to recognize that many psorosis-A isolates are difficult to be mechanically transmitted or perhaps do *not* transmit mechanically from infected sweet orange or citron to other citrus or herbaceous hosts.

Variability of leaf symptoms

A definitive standard for diagnosing psorosis by specific leaf symptoms or patterns may be difficult to achieve. *Leaf symptoms vary widely with isolates and mixtures of isolates.* Different temperatures may induce different leaf symptoms (59, Fig. 1b). Infected leaves of sweet orange, mandarin, lemon, citron, sour orange etc. will show marked differences in symptomatology with different psorosis isolates (30, 48, 74). (Roistacher unpublished) tested 21 isolates of psorosis-A obtained from field trees at the University of California citrus variety collection at Riverside. These were graft-inoculated into seedlings of Dweet tangor, sweet orange and citron, held at temperatures of 24-27 C day and 18-21 C night and observed for symptom development. They were also tested for knife-slash mechanical transmission from citron to citron. There was *much variability* in the symptoms induced by the various isolates. Very few symptoms were the same for different isolates on all three of the indicators. Only two of the 21 isolates transmitted mechanically from citron to citron. However, despite this variability of symptomatology and mechanical transmission, all 21 isolates in sweet orange protected against a challenge from psorosis-B lesion inoculum, thus indicating relationship to psorosis-A.

Over a period of 28 yr, 11 select and diverse isolates of psorosis-A, held in the virus bank at the California Citrus Clonal Protection Program at Riverside were continually evaluated for symptom expression on sweet orange and Dweet tangor indicator seedlings. These 11 isolates in sweet orange protected against a challenge from psorosis-B lesion inoculum indicating relation to psorosis-A. The results of 209 tests showed much variation among the isolates in symptom expression (Table 1). Certain isolates such as

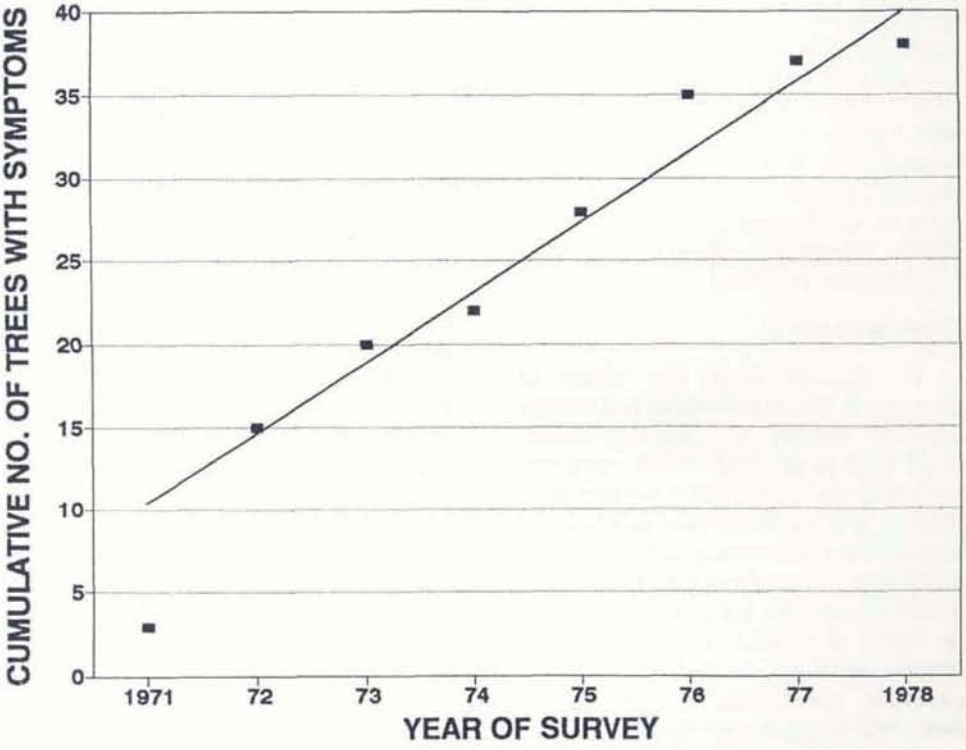

Fig. 1. The natural spread of psorosis in nucellar grapefruit in Texas showing the cumulative number of trees showing symptoms over a 7-yr period. From Timmer and Garnsey (66).

TABLE 1
SYMPTOMS INDUCED ON SWEET ORANGE AND DWEET TANGOR INDICATOR PLANTS BY 11 PSOROSIS SOURCES[a] USED AS STANDARDS OVER A 28-YR PERIOD

Isolate Code	Years under test	No. of tests[b]	SYMPTOM REACTION				
			Negative	Shock reaction	Young leaf	Mature leaf	Yellows
200	27	22	2	5	17	0	2
201	28	25	0	10	20	1	1
202	19	16	1	5	13	2	0
203	19	27	0	21	15	3	2
205	19	20	0	10	16	0	2
208	18	17	1	6	12	1	2
209	18	40	0	27	20	0	4
212	11	14	0	3	14	3	1
213	6	15	2	6	9	7	2
215m	3	8	0	6	6	2	1
216m	2	5	0	4	3	1	0
TOTALS		***209***	***6***	***103***	***145***	***20***	***17***

[a]Sources were held at the virus bank at the University of California Rubidoux facility as part of the California Citrus Clonal Protection Program.
[b]Each test was to one plant per indicator.

Codes ps-209 and ps-203 consistently induced more shock symptoms (68 and 78% respectively of the inoculated plants). In contrast, other isolates such as codes ps-200 and ps-202 induced less shock (23 and 31% respectively of the inoculated plants). Less than 10% of the plants showed mature leaf symptoms and less than 8% showed yellows. Young leaf symptoms were variable and differed with the various isolates, but were present in most tests. In 6 of the 209 tests,no symptoms were observed in young or mature leaves of inoculated seedlings, though inoculating tissue remained alive.

Not all leaf patterns are due to psorosis. Certain genetic conditions may cause a spotting in field trees of sweet orange similar to pin point spots associated with ringspot. This can be differentiated by indexing. Environmental dust and air pollution can cause psorosis-like symptoms on young leaves in the greenhouse . Also spray injury will induce ringspot symptoms on leaves. Spraying with insecticides should be avoided during the crit¡ical period of young leaf development. The presence of a number of non-inoculated control seedlings is essential for differentiating non-psorosis leaf spots from those induced by pathogens.

Supplemental light during the winter months will enhance psorosis-related symptom development and increase the growth of young leaves and should be included in a plant laboratory for indexing (54,65).

FIELD TRANSMISSION OF PSOROSIS

Seed transmission. The transmission of a PLP through the seed of infected Carrizo citrange at rates of 15 to 31% was reported from Florida (15). The photograph of a leaf with symptoms by Childs and Johnson (15) shows mild interveinal leaf flecking similar to that induced by the concave gum pathogen during the first flush of growth and is similar to that of the PLP reported from Spain. (46). The identity of this Florida seed transmitted disease was never further classified as a psorosis-A by cross protection experiments or by observation for development of bark lesions.

Campiglia *et al.* (12) reported that 1% of 250 trifoliate seedlings in the Salto region of Uruguay showed symp-

toms of psorosis and they proposed seed transmission. It is highly probable that this psorosis was due to natural spread in view of the high rates of natural transmission of psorosis known in that region (6,52). Pujol and Beñatena (52) presented a number of arguments against seed transmission of psorosis in Argentina. They observed no young leaf symptoms from many seedlings grown from seed of diseased trees. They cite other observations and definitively rule out seed transmission. However, one year later Pujol (53) presented evidence that oak leaf pattern and flecking was transmitted at 43.7% efficiency through Troyer citrange seeds. Wallace (77) reported on observations for psorosis that he and Fawcett made of approximately 20,000 seedlings grown from seed of psorosis-infected trees and they found no evidence of seed transmission. Definitive evidence for seed transmission of psorosis-A is thus lacking.

Pollen transmission. Vogel and Bové, (73) demonstrated transmission of cristacortis, concave gum and impietratura by placing pollen from flowers of infected trees under the bark of indicator plants and observing young leaf symptoms. Psorosis-A was not tested. However, Navarro (personal communication) observed transmission of psorosis-A when pollen taken from flowers of psorosis-A infected trees was placed under the bark of indicator seedlings. There is no experimental evidence that psorosis can be transmitted via pollination of flowers and through the seed.

Natural transmission of psorosis. The evidence for natural spread of psorosis is convincing, and vector involvement is highly probable. In a survey of all trees in the citrus variety collection at the Citrus Research Center at Riverside, 29 introductions were found infected with psorosis-A. All of the 29 isolates were protected against a challenge with psorosis-B lesion inoculum and were classified as psorosis-A. Fourteen of the 29 introductions were suspected to have been naturally infected since they were either originally introduced as seed, or only one of a pair of trees was found infected. (Roistacher, unpublished).

Timmer and Garnsey (72) showed natural spread of a necrotic ringspot, probably a psorosis B-type isolate in nucellar, virus-free grapefruit trees in Texas. Thirty-five trees became infected over a seven-year period at a rate of about five trees per year as shown in Fig 1. Pujol and Beñatena (52) recorded the presence of psorosis in seedling trees in large numbers in Argentina and observed that the disease was spreading. They suggested that "*psorosis in Concordia is spread by vector, probably a sucking insect*". Beñatena and Pujol (5) concluded that psorosis is disseminated in the nursery by methods other than propagation and suspected vector transmission. The strongest evidence for natural or vector spread of psorosis in Argentina is from an experiment at the I.N.T.A. Experiment Station in Concordia where eighteen rows containing 504 psorosis-free nucellar trees were planted next to four rows containing 90 psorosis-infected trees (6,51). Fig. 2 shows the natural spread of psorosis from the infected to the non-infected trees three and seven years after planting in the field. This study was based on observations of shock and foliar symptoms in the field. No bark scaling could be observed in the young seedlings trees and no glasshouse indexing or challenge inoculation with psorosis-B was done. Note that the spread is greatest (38 infected trees) in the first six rows adjacent to the infected trees compared to only 14 trees found infected in the 12 farthest rows.

ELIMINATION OF PSOROSIS FROM PROPAGATIVE BUDWOOD

Psorosis can be eliminated from citrus budwood by thermotherapy 11, 61) and/or shoot tip grafting (STG) (45, 46, 60, 62). The recommended procedure for thermotherapy is to graft pre-conditioned psorosis infected buds on seedlings of Troyer or Carrizo citrange

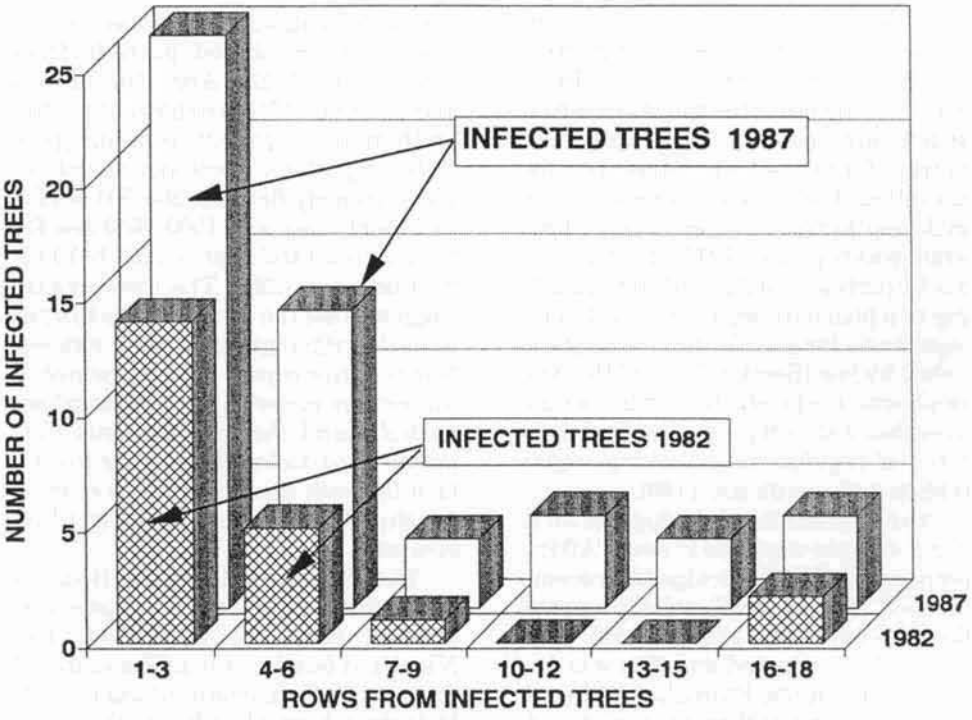

Fig. 2. The natural spread of psorosis in the experiment of Beñatena and Portillo (6). The graph shows the number of trees found infected three and seven years after planting of 18 rows containing 504 nucellar trees adjacent to four rows containing 90 psorosis infected trees (to the left of the graph). Note that the spread is greatest in the first six rows adjacent to the infected trees and diminishes with distance from infection.

and place the grafted plants in a temperature cabinet for 8 to 12 weeks at 40 C for a 16-hr day and 30 C for 8 hr night. Other recommended rootstocks are Rangpur lime and trifoliate orange which were found to be highly tolerant to heat. The sweet orange, rough lemon and certain other rootstocks will not tolerate heat (11).

Some isolates of psorosis are more difficult to eliminate by STG. Roistacher *et al.* (60) shoot tip-grafted four isolates of psorosis-A and achieved 0, 50, 86 and 100% freedom from virus respectively. Psorosis-A isolate ps-209 which gave 0% response to shoot tip grafting was tested again and only 1/11 of the tips were free of virus whereas 12/12 of psorosis-B tips tested virus-free after STG (62). Navarro *et al.* (46) showed that the temperatures at which psorosis-infected plants were grown prior to STG markedly influenced the successful elimination of the pathogen. Shoot-tips from five different psorosis-A sources were: 1) taken from the field; 2) taken from denuded plants (where all leaves were removed to force young shoots) and held in a greenhouse at 18-25 C and 3) taken from denuded plants held in greenhouse at 27-32 C. The number of plants found free of virus after STG was 5/52, 4/31 and 38/60 respectively. In addition to psorosis-A, a psorosis-like-pathogen also responded to pre-conditioning by warm temperatures in the successful elimination of the pathogen. Indexing after shoot-tip grafting and/or thermotherapy is an absolute necessity and STG or thermotherapy alone or in combination is no guarantee that the therapy will eliminate psorosis.

CERTIFICATION AND ERADICATION OF PSOROSIS

The pioneering work of Fawcett (29,30), showing that psorosis was a graft transmissible virus and that it

could remain symptomless in many trees, led to the development of the first certification program for citrus. Fawcett (31) suggested the use of virus-free sources of budwood for the propagation of trees for new plantings based on observations for leaf and bark symptoms in the mother trees. A certification program was begun in 1937 by the California Department of Agriculture according to a plan outlined by Fawcett. The legal basis for certification was established by law (Section 120.5 of the Agricultural Code of California) which provided authority for the establishment of regulations governing registration and certification (40).

The first eradication program for a citrus disease was done in South Africa for psorosis (25,26). Doidge (25) recommended that an eradication program for the elimination of trees with bark lesions be conducted and this was accomplished by the Psorosis Act No. 42 of 1927. Over 6,000 trees were found with scaly bark symptoms and were eradicated (17). However, Marais *et al.* (44) reported that psorosis is still a threat since it is present in old line citrus in South Africa. With the discovery of the seedling index for psorosis by Wallace in 1945 (74), this new and rapid test for determining the presence or absence of psorosis became the standard method for assuring that propagative budwood would be free of the pathogen and the seedling index was incorporated in certification programs worldwide.

STUDIES ON VIRUS PURIFICATION

The 48 kd capsid protein. Derrick *et al.* (22) indicated that citrus ringspot is associated with a unique two component virus with elongated flexuous particles. The top and bottom components separated in a sucrose density gradient contain the same capsid protein of 48 kd. Each component was non-infective when transmitted individually, but infective when mixed together. However, infectivity to *C. quinoa* was found to be difficult and was lost under certain conditions. A number of isolates of ringspot and psorosis from Florida (18, 21, 23), Argentina (18,36) and Spain (18,47) have shown these top and bottom components containing the 48-50 kd protein. These virus particles are extremely flexible, 300-500 nm for the short ones and 1500-2500 nm for the long ones and approximately 10 nm in diameter (22). The researchers suggests that the virus belongs to a yet to be described group of plant viruses: "*the putative capsid protein , which is larger than expected for a filamentous particle, and the very flexuous filamentous particles that appear to contain the split genome are not characteristic of any known group of plant viruses.*"

The 29 kd capsid protein. Bouhida (7) transmitted a virus from psorosis-infected citron to *C. quinoa* and *Nicotiana benthamiana.* The source of the psorosis isolate he used was ps-203-M derived from a Kao Panne Pummelo which was introduced from Thailand into the variety collection at Riverside, California in 1930. Isolate ps-203-M induced severe shock plus young leaf symptoms when bud transmitted to sweet orange and citron. The virus was mechanically transmitted by stem slash from infected citron to citron. This mechanically transmitted psorosis isolate, when graft-inoculated into sweet orange seedlings protected against a challenge from psorosis-B lesion inoculum and was designated as a psorosis-A (63). Bouhida (7) found flexuous rod aggregates in infected cells and flexuous virus particles in the partially purified ps-203-M source. He concluded that "*studies on host range, dsRNA, serological tests and histological observtions suggest that this virus belongs to a new and distinct group*". He was not successful in transmitting these particles back to citron or sweet orange to reproduce the disease.

Levy and Gumpf (43) working with this same psorosis source (ps-203-M) used by Bouhida (7), were able to mechanically transmit a virus to a number of herbaceous hosts. They were also able to dodder transmit the

virus from citron to *Capsicum annum* and reciprocally transmit it back to citron, inducing symptoms in the leaves of citron. dsRNA patterns for the virus were similar when obtained from either infected citron or herbaceous hosts and flexuous rod shaped particles 660-665 x 12 nm were observed in extracts of infected plants. They suggested that the virus belongs to the carlavirus group. A 29-kd protein was identified and they reported that a polyclonal antiserum to the flexuous particles weakley detected ps-203-M and other psorosis strains using ELISA.

In a recent report, Byadgi *et al.* (10) characterized a filamentous virus particle associated with a ringspot disease of citrus which is widespread in India. The disease is graft transmissible but not sap transmissible to *C. quinoa*. They found two types of filamentous particles associated with the disease; "*—the most common were virus-like, 640 nm long and with a clearly seen basil helix, thus resembling capillo-or closteroviruses. Particles of the second type were thinner and did not have any clear modal length; they appeared to be protein aggregates.*" A polyclonal antiserum prepared against the virus-like particles detected the virus in field trees. These virus particles did not react to an antiserum obtained from K. S. Derrick to a Florida ringspot isolate.

RINGSPOT - IS THIS A SEPARATE VIRUS?

Ringspot, as a symptom of psorosis, was first mentioned by Fawcett (28) in 1932 in his original description of the disease. He reported that "*frequently, curious ringspots form on the leaves*". Fawcett (29) and Fawcett and Klotz (32) further described symptoms on leaves associated with psorosis "*—in some cases rounded clear spots are formed on some of the older leaves. These spots vary in size from mere dots to areas 10 to 15 mm in diameter and often are accompanied by a slightly raised brown surface, occasionally in the form of rings*". Fawcett mentioned that the spots on older leaves had been observed for a long time. Also, herbarium specimens of young leaves collected in 1923 from young trees showed small spots and these trees later developed bark scaling. Fawcett (30) further describes ringspot symptoms on fruit in association with bark scaling symptoms. These ringspot symptoms on leaves and fruit were illustrated by Fawcett and Bitancourt (34) and Fawcett and Klotz (35) and by Klotz (41). Ringspot symptoms on leaves and fruit were clearly associated with psorosis bark scaling.

Wallace and Drake (76) first suggested ringspot as a distinct disease based on symptoms they observed on sweet orange seedlings inoculated from a field lemon tree which showed a small lesion on one limb resembling a psorosis bark lesion. Subsequent inoculations to various indicator plants showed a range of severe symptoms of spots, rings and leaf blotching. They indicated that the citrus ringspot virus, was found in trees with psorosis-A but was also found separately. Of significance, they reported that "*Sweet orange plants experimentally infected with ring spot virus were* not *protected when later challenged with psorosis-A lesion inoculum.*" They suggested that the two viruses are not closely related. However, in the same report, Wallace and Drake (76) mentioned that sweet orange seedlings previously infected with psorosis-A from non-lesion inoculum or with blind pocket did not develop ringspot symptoms when later challenged with the ringspot inoculum. Desjardins *et al.* (24) transmitted Wallace and Drake's ringspot source by dodder (*Cuscuta subinclusa*) to a number of citrus species as well as periwinkle and petunia, and back transmitted it to citron by dodder. Wallace and Drake (76) initially designated this as a distinct virus disease because of its symptomatology and its lack of protection when challenged with psorosis lesion inoculum. When this original ringspot source from field 8C, Row 2 Tree 19 was put into the citrus virus bank at Rubidoux

in 1979 and indexed, typical strong ringspot symptoms were induced in grapefruit. Later, a sweet orange seedling inoculated with this source showed complete protection against a challenge with lesion inoculum of psorosis-B indicating that this ringspot source *was* related to psorosis-A.

Timmer (68) used the name "citrus ringspot virus-necrotic strain" to describe a disease found in grapefruit in Texas which showed strong leaf and fruit symptoms and also induced strong leaf symptoms in inoculated plants. Cross protection tests in Mexican lime suggested its relation to psorosis-A. Timmer also reported that he could induce similar symptoms from budwood taken from nucellar trees showing typical psorosis bark lesions. Subsequent papers referred to this strong-reacting disease as the citrus ringspot virus (CRSV) (70, 71, 72). Mechanical transmission to herbaceous hosts was demonstrated for CRSV from both Florida and Texas isolates (37).

Garnsey and Timmer (38) were able to mechanically transmit a ringspot isolate from citron to *C. quinoa*, and then from a single lesion on *C. quinoa* to *G. globosa*. When citron was mechanically inoculated from lesioned *G. globosa* it showed typical ringspot lesions. When sweet orange budlings were graft inoculated from the infected citron, bark lesions were induced in the sweet orange in 9 to 12 months. These results indicated that infectivity present in symptomatic *C. quinoa* is related to psorosis bark scaling.

Studies by Derrick *et al.* in Florida (21, 22, 23), Garcia *et al.* in Argentina (36), Naval-Navas-Castillo *et al.* in Spain (47) and Da Graça *et al.* (18) using Florida, Argentine and Spanish isolates of psorosis or CRSV all showed the top and bottom components containing the specific 48-50 kd capsid protein. They also observed filamentous long and short particles by serologically specific electron microscopy. The flexuous particles were first observed by Derrick *et al.* (21) associated to the CRSV-4 isolate from Florida. Later they could see scattered particles of similar morphology associated to the CRSV-6, also from Florida. Using the antiserum obtained to CRSV-4, Navas-Castillo *et al.* (49) observed scattered particles associated to a Spanish ringspot isolate RS-SR. In a personal communication Navas-Castillo and Moreno reported that they could detect these flexuous particles in psorosis-A and psorosis-B isolates.

Derrick *et al.* (23) reports "*Cross protection tests with various isolates tend to indicate that psorosis-B is a severe form of psorosis-A and the CRSV is similar, if not identical to psorosis-B. This view is supported by our recent findings and at this point it would appear that various isolates of CRSV and citrus psorosis virus are either identical or strains of the same virus. Thus, we consider ringspot and psorosis to be synonymous.*" da Graça *et al.* (18) in analyzing 14 ringspot and psorosis isolates from Florida, Argentina and Spain concluded: "*psorosis and ringspot found in various parts of the world are caused by a virus similar or identical to CRSV-4 and is consistent with previous suggestions that psorosis and ringspot are similar*". Navas-Castillo and Moreno (48) concluded that six of eight ringspot isolates collected from symptomatic trees in Spain could not be distinguished from psorosis on the basis of greenhouse symptoms in indicator plants, cross protection, mechanical transmission to *C. quinoa*, and the presence of the 48 kd band.

The distribution of CRSV in grapefruit has been reported as irregular (71). Some isolates in Texas grapefruit were inconsistently transmitted from the twig bark of field trees to indicator plants, and they were transmitted only from areas showing bark lesions on two grapefruit trees . "*This isolate was irregularly transmitted even within symptomatic leaves and could not be transmitted to C. quinoa.*" Similarly, in Israel, Bar-Joseph and Ben-Shalom (4) showed limited spread of impietratura and psorosis-A in grapefruit. The viruses remained localized eight years after inoculation to limbs of field trees.

It is conceivable that in Wallace and Drake's initial definition of ringspot (76), the tissue they used for cross protection could have been void of the virus whereas other ringspot tissue, when used as challenge inoculum against psorosis-A contained the virus, explaining the differences they observed. Based on the evidence of recent research, and to avoid confusion, ringspot should not be designated as a separate virus but as a severe form of psorosis.

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