CHAPTER 1

Psorosis and Related Diseases

Recent Developments in the Citrus Psorosis Diseases

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THE NAME *psorosis* has been used to designate a group of virus diseases namely psorosis A, psorosis B, blind pocket, concave gum, crinkly leaf, and infectious variegation, which Fawcett (7), Fawcett and Klotz (6), Fawcett and Bitancourt (5), and Wallace (17, 18) accepted as being caused by related virus strains. This conclusion was based on the fact that the type sources of these diseases, maintained by Fawcett and later studied by Fawcett, Bitancourt, and Wallace, all caused similar patterns on young leaves of citrus. This reaction became known as the psorosis young-leaf symptom. However, the diseases were distinguished from each other on the basis of the other symptoms which they caused on infected citrus trees. The disorders will not be described individually in detail since they are well illustrated in the papers mentioned.

In 1957, Wallace (17) concluded that psorosis B, as described by Fawcett, is merely an early, severe, general reaction of healthy sweet orange [*Citrus sinensis* (L.) Osb.] to inoculation with a piece of bark from bark lesion of psorosis A. However, when healthy trees are infected from tissue grafts of non-lesion bark, the typical young-leaf symptoms of psorosis A develop and are followed by localized bark lesions several years later.

Sweet orange seedlings inoculated with non-lesion tissue (bark patches, buds, twig grafts, or leaf patches) previously (17), failed to develop psorosis-B symptoms when reinoculated with psorosis lesion bark. This protective reaction was later used to ascertain whether the viruses of concave gum, blind pocket, crinkly leaf, and infectious variegation are related to psorosis-A virus. In these studies the virus source material traced back to the original "cultures" studied by Fawcett, and all caused the characteristic leaf flecking of young leaves. When sweet orange seedlings previously inoculated from these stock sources of concave-gum, blind-pocket, crinkly-leaf, and infectious-variegation viruses were reinoculated, using psorosis-A bark lesion inoculum, they failed to develop the early, severe reaction that such inoculum normally causes on healthy trees. However, protection by crinkly-leaf virus was not as complete or as consistent as with the other virus sources (17).

There is now some indication that inclusion of at least some of these four diseases in the psorosis group on the basis of common young-leaf symptoms and cross-protection reactions may have resulted from the presence of a contaminating virus. Previously reported studies dealing with the relationship between the so-called psorosis types are discussed in this paper together with some unpublished data obtained recently by the author. New information on seed transmission of psorosis viruses and the latest available information relative to mechanical transmission and purification of these viruses are reviewed.

New Information on Relationships

CRINKLY LEAF AND INFECTIOUS VARIEGATION.—In Australia, Fraser (9) reported that numerous lemon [*C. limon* (L.) Burm. f.] trees display the characteristic mature-leaf symptoms of crinkly-leaf virus as described by Fawcett and Bitancourt (5) and Wallace (17, 18). Inoculations from such trees to seedlings of lemon and other citrus varieties resulted in the early appearance of many small, clear, circular spots, especially on young leaves of Lisbon and Eureka lemon. As affected leaves reached maturity, they became crinkled, the same as field tree leaves. Fraser stated that young-leaf symptoms of psorosis failed to develop on seedlings inoculated from Australian sources of crinkly-leaf virus and that seedlings infected with crinkly-leaf virus developed characteristic oak-leaf patterns when later inoculated with psorosis-A virus. From this reaction, it is clear that concave-gum virus was present in the inoculum used in these tests.

Perhaps the failure of the crinkly-leaf virus to inhibit the development of oak-leaf patterns is of no particular significance. However, the com-

plete absence of young-leaf flecking of psorosis in plants inoculated from the crinkly-leaf sources is of interest because this is the basis of Fraser's (9) suggestion that inclusion of citrus crinkly-leaf diseases in the psorosis group by California workers resulted from the use of inocula carrying both crinkly-leaf and psorosis-A viruses. This author, pursuing that suggestion further, performed additional studies on the so-called psorosis viruses, particularly psorosis A, crinkly leaf, and infectious variegation. Crinkly-leaf virus from certain field sources in California is mixed with other psorosis viruses. One of these, psorosis A, or possibly blind pocket, causes the typical young-leaf flecking originally described by Fawcett. The other virus causes the spotting described by Fraser and the characteristic persistent crinkle of mature leaves. When sweet orange seedlings infected with such a virus mixture are later inoculated with patches of bark from psorosis-A lesions, they are protected against development of early bark lesions, mature-leaf symptoms, and twig dieback. Some other sources of virus from crinkly-leaf-affected trees occasionally induced slight leaf flecking, but regularly caused the leaf spotting and crinkly leaf. In sweet orange these viruses provided no protection against bark-lesion inoculum of psorosis A.

Similar studies with the sources of infectious-variegation virus studied earlier (17) showed that they too were mixed with other viruses. In addition to the virus causing variegation, another leaf-flecking virus was present. This latter virus was screened out by mechanical transmission, leaving only the virus that caused variegation. The mixture of viruses protected sweet orange against psorosis-A bark-lesion inoculation, but the mechanically transmitted infectious-variegation virus did not. Furthermore, the isolated infectious-variegation virus induced early leaf spotting identical with that associated with crinkly-leaf infections.

The circular spotting described by Fraser (9) has been encountered many times in California, where it was called pin-point spotting. However, the symptoms were not associated with a specific virus prior to Fraser's observations. Since the individual spots on affected leaves are very small, pin-point spotting seems more descriptive than circular spotting.

In California, crinkly-leaf virus from one source was transmitted through a lemon seed. The infected seedling came from a lemon tree that showed both crinkly-leaf symptoms and psorosis-A type of leaf flecking. The virus mixture from the parent tree protected sweet orange against psorosis-A lesion inoculation, but the seed-transmitted virus did not. This latter component of the mixture caused pin-point spotting and crinkly

leaf on lemon leaves, but did not consistently induce typical psorosis leaf flecking. Upon occasion, however, one or two young leaves of sour orange (*C. aurantium* L.) and *C. pennivesiculata* (= *C. moi*) developed apparently typical psorosis leaf flecking after inoculation with the seed-transmitted virus. Furthermore, C. N. Roistacher (unpublished) observed that seedlings of Dweet (*C. sinensis* x *C. reticulata*) inoculated with this same virus and given 5 hours additional artificial light daily thereafter developed characteristic psorosis leaf flecking.

Subsequent studies (Wallace, unpublished) showed that this California source of crinkly-leaf virus, which is not mixed with psorosis-A, concavegum, or blind-pocket viruses, can cause typical psorosis young-leaf flecking on several citrus varieties. A culture of the seed-transmitted crinkly-leaf virus, after purification by Dauthy and Bové (3, 4), was obtained for comparison with the original virus which had been maintained in California by graft transfer. Both sources of virus caused varying amounts of young-leaf symptoms when inoculated into 12 kinds of citrus. Leaf flecking was especially striking on Dweet tangor and sour orange, but was clearly evident also on some leaves of Madam Vinous sweet orange, some mandarin varieties, and *C. excelsa*. During April and May, conditions in the greenhouses seemed particularly favorable for the development of this leaf symptom. Both before and after purification, this source of crinkly-leaf virus caused strong pin-point spotting and typical crinkly-leaf symptoms on Eureka lemon. Whether or not the spotting differs from that described in Australia by Fraser (9) has not been determined.

Other unpublished studies by the author indicate that the viruses of crinkly leaf and infectious variegation appear to be closely related. This evidence results from two reactions. Eureka lemon and several other kinds of citrus that show strong symptoms of infectious variegation have on numerous occasions produced vigorous shoots that showed only pin-point spotting and mild crinkly-leaf symptoms. Transfers from such growth gave only these symptoms of crinkly leaf. However, plants propagated from the recovered shoots and later inoculated with infectious-variegation virus remained unaffected. Similarly, lemon and sour orange seedlings experimentally infected with the seed-transmitted and/or the mechanically transmitted crinkly-leaf virus were completely protected against challenge inoculations with infectious-variegation virus.

Whether or not all virus sources that cause infectious variegation also contain crinkly-leaf virus has not been determined. Only two naturally occurring field sources have been studied in California and both pro-

duced "recovered" growth that yielded only crinkly-leaf virus. Of course, there is the possibility that crinkly-leaf virus arises as a mutation of infectious-variegation virus or vice versa. Whatever the origin of the crinkly-leaf virus in plants with infectious variegation, it not only suppresses symptoms of infectious variegation in the recovered shoots, but apparently prevents the infectious-variegation virus from becoming established in them. Furthermore, in no instance has infectious-variegation virus been obtained from lemon seedlings first inoculated with crinkly-leaf virus and later inoculated with infectious-variegation virus. This remained true whether tests for the presence of infectious-variegation virus in the double-inoculated plants were made by tissue-graft transfer or by mechanical transmission.

CONCAVE-GUM VIRUS.—Roistacher and Calavan (16) reported that virus isolates from several foreign sources and one California source caused oak-leaf patterns on indicator plants, but did not protect sweet orange against psorosis-A bark-lesion inoculations. This means that seedlings infected with this virus, presumably concave gum, developed early general bark lesions when subsequently inoculated with psorosis lesion bark patches. On the other hand, Wallace (17) consistently obtained protection by a field source of concave-gum virus not tested by Roistacher and Calavan (16). This particular source of concave-gum virus is still available and studies are now in progress to determine if it is contaminated with psorosis-A virus. If this proves to be the case, that would explain the protection observed in earlier investigations (17).

On the basis of the results of Roistacher and Calavan (16) and pending further study, concave-gum virus appears unrelated to psorosis-A virus. Although the failure of concave-gum virus to protect against psorosis-A virus is not absolute proof of non-relationship between the two viruses, it suggests non-relationship, or at least that the relationship is not as close as reported (17).

SEED TRANSMISSION.—Seed transmission was not detected in extensive tests with psorosis A (scaly bark) in California. Wallace (17) mentioned two cases of transmission of crinkly-leaf virus through lemon seeds. In Argentina, Pujol and Benatena (13) and Pujol (14) believed that natural transmission of psorosis occurred somewhat commonly, but that they demonstrated this conclusively is not clear. The wide range of symptoms described by Pujol (14), as well as the illustrations shown, leave some doubt that the reactions reported are caused by psorosis virus. This is true of Foto No. 1 in the publication cited above, which shows a leaf with symptoms of citrus ringspot, such as is described in this volume by

Wallace and Drake. This virus causes blotches, small rings, and stem necrosis or shock effects such as Pujol (14) described on affected plants in Argentina. Although it was reported that virus from naturally infected plants protected sweet orange seedlings against psorosis-A bark-lesion inoculation, no further data were presented, nor was it reported that this inoculum gave the expected positive reaction on healthy control plants. This study was concerned with a virus or viruses that are spread naturally, but it was not clearly established whether spread is by vectors or through seeds. Also, there is still some question of the identity of the causal virus.

Bridges *et al.* (1) found a leaf-flecking form of psorosis in nursery trees on Carrizo citrange (*C. sinensis* x *Poncirus trifoliata*) rootstock propagated from psorosis-free sweet orange in Florida. Childs and Johnson (2) established the fact that one of four Carrizo trees at the United States Department of Agriculture (USDA) Orlando Station, the sources of all the Carrizo seedlings in Florida nurseries, was infected with psorosis and that seeds from that tree transmitted the virus at levels of 15 to 31 per cent, depending on the variety of test plant used. Pujol (15) reported transmission of psorosis virus through seeds from a psorosis-infected Troyer citrange, finding 7 diseased of the 16 seedlings tested. The illustration of leaves of sweet orange inoculated with tissue from Troyer seedlings clearly shows that the virus was that of concave-gum disease.

Since Troyer and Carrizo citrange are closely related, these developments suggest that seed transmission may be a varietal characteristic and emphasize the necessity of selecting virus-free sources of rootstock seed.

MECHANICAL TRANSMISSION.—Except for several reports of mechanical transmission of infectious-variegation and crinkly-leaf viruses from citrus to citrus and to some herbaceous hosts, no reports have been published of transmission of other viruses of the psorosis group. In limited tests, Wallace and Drake (unpublished) failed to transmit mechanically the viruses of psorosis A, concave gum, or blind pocket. However, Weathers (unpublished) infected four Rangpur lime seedlings with sap from a tree showing leaf symptoms typical of psorosis A. The virus has not been positively identified, but it is either psorosis A or blind pocket.

In their first report of sap transmission of infectious-variegation virus, Grant and Corbett (11) stated that plants of sour orange and Duncan grapefruit (*C. paradisi* Macf.) inoculated and maintained at greenhouse temperatures of 20-21°C developed infectious variegation only. However, at higher temperatures, inoculated plants sometimes exhibited non-per-

sistent vein banding, vein accentuation, and chlorotic blotches which Grant and Corbett considered to be the young-leaf symptoms of psorosis. These authors concluded that more than one virus or virus strain was present in their source of infectious variegation. Earlier, Grant and Smith (10) stated that this virus source caused typical psorosis symptoms on calamondin. Thus, it appears that Grant and Corbett (11) worked with a mixture of infectious-variegation and psorosis-A viruses. If that is so, they apparently succeeded in transmitting psorosis-A virus mechanically in mixture with infectious-variegation virus. In the writer's attempts to mechanically transmit known mixtures of infectious-variegation and psorosis-A viruses in California, the latter virus was always screened out.

ISOLATION OF CRINKLY-LEAF AND INFECTIOUS-VARIEGATION VIRUSES.— The first report of isolating either of these viruses appears to be that of Dauthy and Bové (3). In a partially purified preparation of crinkly-leaf virus obtained by density gradient centrifugation, these authors observed virus-like particles measuring approximately 14 mμ in diameter. Majorana and Martelli (12) also used density gradient centrifugation for purification of crinkly-leaf virus, and their electron microscope preparations revealed isodiametric particles measuring approximately 26 mμ. In this volume, Dauthy and Bové, and Martelli, Majorana, and Russo agree that the diameter of the crinkly-leaf virus particle is approximately 26 or 27 mμ. The first two authors also state that preparations of infectious-variegation virus yielded particles having the same diameter as particles of crinkly-leaf virus. Thus, it appears that purification and characterization of these viruses is progressing. Such information for the other so-called psorosis viruses should aid in determining their relationships to crinkly-leaf and infectious-variegation viruses and to each other.

Discussion and Conclusions

There is need for further investigation of mechanical transmission of the viruses of psorosis A, blind pocket, and concave gum. At present, failure to transmit these viruses mechanically in most trials, and the ease of transmitting infectious-variegation and crinkly-leaf viruses both support the idea that the latter two do not belong in the psorosis group. As pointed out previously, recent information on concave-gum virus suggests that it may not be related to psorosis-A virus. Whether or not psorosis-A and blind-pocket viruses are related has not been fully investigated. There is no simple way of determining if blind-pocket-infected field trees

are not also infected with psorosis A as long as such doubly infected trees display no bark lesions of psorosis. In California, orchard trees are commonly found with both blind pockets and psorosis-A bark lesions, and we have assumed that blind-pocket virus alone does not cause bark lesions. This was assumed because certain trees 25 years of age or older show only blind pocket. If such trees were infected with psorosis-A virus one would expect bark lesions to have developed by that time. In some of Fawcett's early experiments, seedling sweet orange trees were inoculated with blind pocket. These trees have shown young-leaf flecking and have developed some blind pockets, but no bark lesions after 28 years. However, trees inoculated simultaneously with the same sources of blind pocket and with a separate source of psorosis A developed bark lesions within 10 years and were severely affected after 28 years. The sources of blind-pocket virus which presumably are not contaminated with psorosis-A virus, are now being further tested to learn whether they will protect against a challenge inoculation with bark lesion inoculum of psorosis A.

The previously reported (17) protection against psorosis lesion inoculum in sweet oranges carrying concave-gum, crinkly-leaf, and infectious-variegation viruses is now believed to have resulted from psorosis-A virus mixed with these three viruses in the inoculum used in the cross-protection tests. However, lack of protection by uncontaminated sources of these three viruses does not prove conclusively that they are not related to psorosis A. In the author's opinion, occurrence under some conditions of typical psorosis leaf-flecking on plants infected with uncontaminated crinkly-leaf virus is sufficient evidence for continuing to include crinkly leaf in the psorosis group. Finally, a close relationship between crinkly-leaf and infectious-variegation viruses now seems to be well established.

Literature Cited

1. Bridges, G. D., Youtsey, C. O., and Nixon, R. R. 1965. Observations indicating psorosis transmission by seed of Carrizo citrange. Proc. Florida State Hort. Soc. 78: 48-50.
2. Childs, J. F. L., and Johnson, R. E. 1966. Preliminary report of seed transmission of psorosis virus. Plant Disease Reptr. 50: 81-83.
3. Dauthy, D., and Bové, J. M. 1964. Isolement du virus de la Frisolée des citrus (crinkly-leaf virus). 1. Note préliminaire. Fruits 19: 187-193.
4. Dauthy, D., and Bové, J. M. 1965. Experiments on mechanical transmission of citrus viruses, p. 250-253. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
5. Fawcett, H. S., and Bitancourt, A. A. 1943. Comparative symptomatology of psorosis varieties on citrus in California. Phytopathology 33: 837-864.

6. FAWCETT, H. S., and KLOTZ, L. J. 1938. Types and symptoms of psorosis and psorosis-like diseases of citrus. (Abstr.) Phytopathology 28: 670.
7. FAWCETT, H. S. 1939. Psorosis in relation to other virus-like effects on citrus. (Abstr.) Phytopathology 29: 6.
8. FAWCETT, H. S., and KLOTZ, L. J. 1939. Infectious variegation of citrus. Phytopathology 29: 911-912.
9. FRASER, LILIAN. 1961. Lemon crinkly leaf virus, p. 205-210. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
10. GRANT, T. J., and SMITH, P. F. 1960. Infectious variegation of citrus found in Florida. Plant Disease Reptr. 44: 426-429.
11. GRANT, T. J., and CORBETT, M. K. 1961. Mechanical transmission of infectious variegation virus in citrus and noncitrus hosts, p. 197-204. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
12. MAJORANA, G., and MARTELLI, G. P. 1965. Il fagiolo quale ospite erbáceo del virus della "variegaturea infettiva" degli Agrumi. Phytopathologia Mediterraneo 4: 170-172.
13. PUJOL, A. R., and BENATENA, H. N. 1965. Study of psorosis in Concordia, Argentina, p. 170-174. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
14. PUJOL, A. R. 1966. Difusion natural de psorosis en plantas citricas. INTA, Estación Exp. Agropecuaria Concordia Serie Technica No. 8.
15. PUJOL, A. R. 1966. Transmission de psorosis a traves de la semilla de citrange Troyer. INTA, Estación Exp. Agropecuaria Concordia Serie Technica No. 10.
16. ROISTACHER, C. N., and CALAVAN, E. C. 1965. Cross-protection studies with strains of concave gum and psorosis viruses, p. 154-161. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
17. WALLACE, J. M. 1957. Virus strain interference in relation to symptoms of psorosis disease of citrus. Hilgardia 27: 223-246.
18. WALLACE, J. M. 1959. A half century of research on psorosis, p. 5-21. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.