Viruses from Rusts and Mildews

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Supported by Grant No. GB25122 from the National Science Foundation.

The assistance of Galen Ashcraft, Dianne De Lisle, A. H. Gold, Conrad Krass, and Robley Williams in the electron microscopy; and of R. Lovie and G. Faccioli in the virus purifications, is gratefully acknowledged.

Accepted for publication 5 March 1973.

ABSTRACT

Particles resembling tobacco mosaic virus (TMV) were found in dip preparations of five species of rusts (Uredinales) and in two species of powdery mildews (Erysiphaceae), but were not found in seven other rusts and five other mildews. For three of these five rusts, similar rods were found associated with germinating uredospores in vitro. Virus-like infections were transmitted to, and recovered from, *Chenopodium quinoa* by 13 rusts and three mildews. Of the positive cases for TMV rods and transmission to *C. quinoa*, the most consistent and most studied were *Uromyces phaseoli* on *Phaseolus vulgaris* cultivar 'Pinto' (bean rust) for the rusts, and *Erysiphe graminis* on *Hordeum vulgare* cultivar 'Mariot' (barley mildew) for the mildews. Electron microscopy was reasonably consistent for different independent collections and observations, and the number of rods per unit of rusted bean tissue was about one four-thousandth of the number in systemically infected tobacco. Most attempts to produce virus-like infections by inoculation with spores, or with juice preparations of rusted or mildewed tissues were unsuccessful, even for bean rust and barley mildew. The TMV-like infections usually had a wide host range but, with two exceptions, were less virulent on tobacco than ordinary TMV. Most isolates became systemic in *C. quinoa*, whereas ordinary TMV did not. One nonTMV-like infection was recovered from *Uromyces polygoni* on *Polygonum aviculare* (knotweed rust). This virus was transmitted only to *C. quinoa* and had a half-life of only 7 sec at 55 C.

Phytopathology 63:1111-1115.

Evidence of viruses in rusts (11) and powdery mildews (7, 10, 11) has been briefly reported. Considerable further evidence has been obtained by F. Nienhaus and K. Yora (*personal communication*). This is a more comprehensive report of virus-like infections in several additional rusts and mildews. It is a summary of evidence that viruses or virus-like entities exist, not an investigation of the nature of these viruses.

MATERIALS AND METHODS.—Most rusts (always in their uredinal stage) and powdery mildews (always in their conidial stage) were collected as natural infections in the San Francisco Bay Area, but *Uromyces phaseoli* on bean and *Erysiphe graminis* on barley had been maintained in the greenhouse for about 30 yr. *Uromyces phaseoli* var. *vignae* was received from Michele Heath.

For electron microscopy, screens were prepared by Brandes' (4) dip method, shadowed with palladium, and examined in an RCA-EMU-3F. For quantitative comparison with ordinary tobacco mosaic virus (TMV), identical amounts of leaf tissue were studied, and particle counts were made on a 1,000 μ^2 area.

For testing virus-like infectivity, two basic methods were used. In one, *C. quinoa* plants with 5-15 leaves, 2-cm or more in length, were inoculated heavily with spores of the test fungus. Rust-inoculated plants were sprayed with a spore suspension and incubated overnight in a moist chamber to favor infection, or sometimes longer to induce large lesions. Mildew-inoculated plants were dusted with dry spores and left on the dry greenhouse bench for infection, or sometimes in a dry dark chamber to induce larger lesions. Some of the lesions which seemed most likely to be caused by a virus were assayed as juice suspensions by further inoculations on *C. quinoa*, and only if these latter plants showed virus-like lesions, was the original inoculation considered a valid transmission of a virus-like infection by spores.

In the other method, fungus-infected tissue and noninfected tissue was separately ground and used as inoculum on *C. quinoa*. If the fungus-infected tissue yielded virus-like lesions, and the nonrusted tissue did not, this was evidence that the fungus contained a virus.

Some assay inoculations, especially of stem and hypocotyl lesions on bean induced by bean rust infection of the primary leaves, were by brush extraction (9). A few trials were made with inoculum filtered through Sephadex (7).

To determine the half-life of a virus, infected tissues of *C. quinoa* were held in water at known temperature for measured intervals, ground in water, and used as inoculum. The half-life was the time for the infectivity to be reduced to one-half that in the untreated controls.

RESULTS.—*Electron microscopy.*—Gross results are summarized in Table 1 and a specimen of rusted bean tissue with more than the average number of TMV-like rods is shown in Fig. 1-A. Not all rod-shaped particles were as straight as typical TMV, and many particles appeared to be pieces of what were once larger particles. Crystals were never seen, but small clusters of rods were common. Rods were found in five out of 12 species of rusts and in two out of six species of mildews. Table 2 shows where TMV-like rods were seen when different parts of rusted bean and mildewed barley were examined. Over an area of 1,000 μ^2, an average of 14 rods were found in a 1:20 dilution of an extract from rusted bean tissue compared to 667 rods in a 1:2,000 dilution of a preparation from systemically infected

TABLE 1. Evidence of viruses in rusts and powdery mildew fungi; observation of particles resembling tobacco mosaic virus (TMV), and passage of the infective agent through *Chenopodium quinoa*.

Fungus species	Host species of fungus	TMV-like rods observed	Recovery of infection from inoculated *C. quinoa*
Coleosporium asterum Syd.	*Aster chilensis* Nees.	+	+
C. madiae Cke.	*Madia sativa* Mol.	+	+
Erysiphe graminis DC.	*Hordeum vulgare* L.	+	+
E. graminis DC.	*Avena sativa* L.	+	[a]
E. polygoni DC.	*Phaseolus vulgaris* L.	–	+
E. cichoracearum DC.	*Baccharis pilularis* DC.	–	[a]
Frommea obtusa (Strauss) Arth. var. *duchesneae* Arth.	*Duchesnea indica* (Andr.) Focke	[a]	+
Kunkelia nitens (Schw.) Arth.	*Rubus vitifolius* Cham. & Schlecht.	–	+
Melampsoridium alni (Thüm) Diet.	*Alnus oregana* Nutt.	–	–
Microsphaera alni DC. ex Wint.	*A. oregana* Nutt.	–	–
Phragmidium sp.	*Rosa* sp.	–	+
Phyllactinia corylea Pers. ex Karst.	*Platanus acerifolia* (Ait.) Willd.	–	+
Puccinia cirsii Desm.	*Cirsium vulgare* (Savi) Tenore	[a]	–
P. evadens Harkn.	*Baccharis pilularis* DC.	–	[a]
P. graminis Pers.	*Holcus lanatus* L.	[a]	–
P. hieracii (Schum.) Mart.	*Taraxacum officinale* Weber	[a]	–
P. iridis (DC.) Wallr.	*Iris xiphioides* Ehrh.	[a]	+
P. oxalidis (Lér.) Diet. & Ell.	*Oxalis Pes-caprae* L.	–	+
P. pelargonii-zonalis Doidge	*Pelargonium domesticum* Bailey	–	+
Sphaerotheca lanestris Harkn.	*Quercus agrifolia* Née.	+	+
Uncinula salicis DC. ex Wint.	*Salix* sp.	–	[a]
Uromyces phaseoli (Pers.) Wint. var. *typica* Arth.	*Phaseolus vulgaris* L.	+	+
U. phaseoli (Pers.) Wint. var. *vignae* (Barcl.) Arth.	*Vigna sinensis* (Torner) Savi	+	+
U. fabae (Pers.) d By.	*Vicia faba* L.	+	+
U. polygoni (Pers.) Fekl.	*Polygonum aviculare* L.	–	+

[a] No observation or no trial.

TABLE 2. Occurrence of TMV-like rods on rusted bean and mildewed barley

Type of specimen	Positive for TMV-like rods	No. of specimens
Bean rust (*Uromyces phaseoli*)		
rust pustules (4-30 day old)	46	48
rust pustules (3-day old)	0	1
chlorotic halo around pustules	4	6
nonrusted area of laminae bearing rust infections	0	5
petiole lesions of rusted bean	1	8
stem lesions of rusted bean	1	9
hypocotyl lesions of rusted bean	5	6
roots of rusted bean	8	11
crushed germinated uredospores	10	10
nonrusted bean	0	6
Barley mildew (*Erysiphe graminis*)		
mildewed barley tissue	17	19
barley mildew conidia	3	4
nonmildewed tissue of barley leaves	1	5
nonmildewed barley	0	1

tobacco tissue. Thus, we estimated the number of rods in systemically infected tobacco to be 4,000-5,000 times as great as in rusted bean. The number of rods from 48 dip preparations of bean rust pustules ranged from 0 to 74 per 1,000 μ^2 field.

Serology.—Serological reactions with standard TMV antiserum were consistently negative with crushed germinated bean rust spores and clarified rusted bean tissue used directly as antigen, but positive with purified virus from rusted bean tissue and with leaf tissue of *Chenopodium amaranticolor, C. quinoa, Phaseolus vulgaris* var. Pinto, *Vigna sinensis* var. 'Black Eye', and *Nicotiana tabacum* infected with the virus from bean rust. These positive serological reactions of lesion tissue with TMV antiserum were correlated with the electron microscopic observation of large numbers of TMV-like rods.

Transmission.—Virus infectivity of uredospores or conidia or fungus-infected tissue was tested for 18 rust, and five mildew, pathogens. Most trials and most successes were with *U. phaseoli, E. graminis*, and *Sphaerotheca lanestris*, and only quantitative results with these species are reported here. For these three species, assays were made at times on *C. quinoa, C. amaranticolor, Cucumis sativus, P. vulgaris, Vicia faba, V. sinensis, Nicotiana glutinosa, N. tabacum*, and *N. clevelandii*. Most were made, however, on *C.*

quinoa because it seemed the best indicator host. Only results on *C. quinoa* will be presented.

Hypersensitive-like and/or virus-like lesions, varying greatly in size, usually appeared in 4 days. Some extreme cases are illustrated in Fig. 1. To date, it has not been possible to determine by inspection whether the lesions resulting from inoculation with fungi would yield virus-like lesions on re-assay to *C. quinoa*. Large lesions sometimes failed to yield virus-like lesions on re-assay, and some small lesions yielded juice-transmissible infections. But large lesions on *C. quinoa*, especially those which appeared on the petiole or which ran down the petiole from the lamina, most often produced virus-like lesions on re-assay, and were most likely to contain virus-like particles visible in the electron microscope.

In 208 tests of infectivity using 954 assay plants, virus was recovered (at least one assay lesion) from

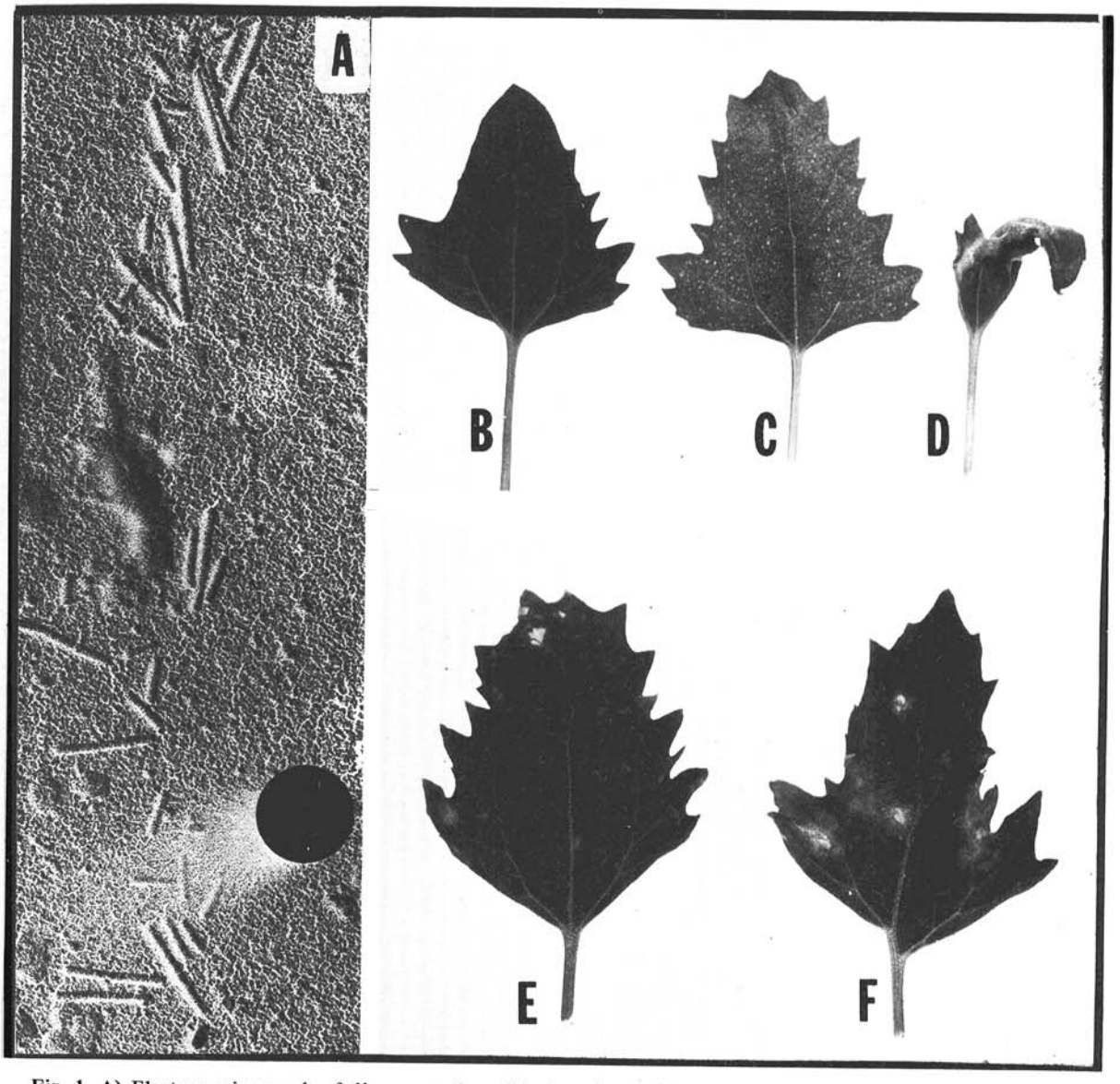

Fig. 1. A) Electron micrograph of dip preparation of lamina of rusted bean. The circular polystryene ball is 264 nm in diameter. This is a greater concentration of TMV-like rods than usually found. Note that the lengths of many rods are less than 300 nm. **B, C, D)** *Chenopodium quinoa* leaves from same trial. B is a noninoculated control. C and D were inoculated with conidia of *Erysiphe graminis*. C shows the hypersensitive-like lesions which commonly result from powdery mildews on *C. quinoa*. D shows a severe case of injury due to *E. graminis*. No virus-like infectivity was recovered from these leaves. Inoculated 15 February, photographed 22 February. **E, F)** *C. quinoa* leaves inoculated with *Uromyces polygoni* on 28 April, photographed 10 May. E was from plant incubated overnight in a moist chamber at inoculation and then left on greenhouse bench. F was from plant given an additional 48 hr in the dark at 6 days after inoculation. Virus-like infections were transmitted from these leaves.

rusted bean tissue 29 times, from *C. quinoa* inoculated with uredospores nine times, from mildewed barley tissue five times, from *C. quinoa* inoculated with barley mildew conidia 12 times, from mildewed oak tissue five times, and from *C. quinoa* inoculated with oak mildew conidia nine times. Total recovery was thus 69 out of 954 or 7.2%. This appears much less than the 19 successes out of 65 trials reported earlier (11), where the trial rather than the assay plant was the basis of comparison. These 69 successes include some cases with about a hundred lesions on a single leaf. In addition to the 954 assay test plants, there were 85 control plants inoculated with supposedly healthy tissue, and on these a total of 10 lesions appeared on six plants, never more than one per leaf.

The most impressive successful transmission in a single test was with uredospores of *Uromyces polygoni* sprayed onto *C. quinoa*. In one plant, part of which was heated 10 sec at 55 C before inoculation, 340 lesions appeared. No TMV-like rods were found in these lesions; however, juice from these lesions produced many further lesions on *C. quinoa*, but on no other indicator plant tested. Plants held in the dark for 1, 2, and 3 days at 6 days after inoculation produced progressively larger lesions than plants maintained in the ordinary greenhouse environment (Fig. 1-F). In contrast to the TMV-like virus from bean rust, which had a half-life of about 3 sec at 99 C, the virus from knotweed rust had a half-life of only 7 sec at 55 C. This virus initially became systemic in *C. quinoa*, but apparently lost this character on continued passage. No infectivity was recovered from the roots of *C. quinoa* plants with systemic symptoms in the tops.

Several, but not all, of the TMV-like isolates produced systemic symptoms in *C. quinoa* and *C. amaranticolor*, whereas ordinary TMV did not. This tendency to become systemic in *C. quinoa* was increased by holding plants in the dark for 1-3 days after inoculation.

Several of the TMV-like isolates produced pits on the lower surface of cucumber cotyledons which had been inoculated on their upper surfaces. The form from snapdragon rust produced systemic symptoms in cucumber in one test.

All rusts tested (except bean rust) produced small to large necrotic lesions on Pinto bean leaves which were heated 3-8 sec at 55 C before inoculation with uredospores, whereas lesions did not usually appear on unheated leaves. Virus was recovered only three times from such virus-like or hypersensitive-like lesions. Several isolates produced systemic symptoms in bean following mechanical inoculation with extracts from these infections, but most produced no lesions or only local lesions, and sometimes these latter were formed only if the leaves had been heated before inoculation.

Quantitative comparisons between different rusts or between rusts and mildews as to virus content were not made, but the most promising source of virus for electron microscopy seemed to be medium to old infections of *U. phaseoli* on the lamina of primary bean leaves. The most promising source of virus by transmission with uredospores appeared to be *U. polygoni*. No consistently successful method of isolating an infective virus from rusts or mildews or from rusted or mildewed tissue has been devised, but the most promising seemed to be to dust conidia of *S. lanestris* on leaves of *C. quinoa*.

The apparent presence of TMV-like rods in rusted bean, broad bean, and snapdragon, as well as mildewed barley, was greatly reduced by heating the donor leaf tissues 1-24 hr before making the leaf dip preparations. The number of rods in rusted bean tissue heated 20 sec at 50 C, averaged only 83% of the number in unheated tissue in six independent trials. This same heat dosage caused no damage to the host and had no apparent effect on the number of rods in tobacco systemically infected with TMV. The significance of this heat effect is not clear.

DISCUSSION.—Considering the abundance of viruses in certain other fungi (6) and in many bacteria, the presence of viruses in rusts and mildews is not surprising. What is more surprising is that they were discovered so late, when they were sought for so early (1). We believe the reason these viruses were discovered so late is that these fungi were previously investigated as vectors of viruses, not primarily as carriers of viruses. It is similar to the discovery of viruses not known to be associated with any natural plant diseases in leafhoppers (2) and in humans. No naturally occurring disease associated with any of the virus infections of this study is yet known, and trials of rusts and mildews as vectors of known natural virus infections have all been negative. Transmission of TMV in oak (12) by *Sphaerotheca* is uncertain.

The relative abundance of virus particles in certain rusted and mildewed tissues, and the failure of previous investigators to notice them in rusted (5) and in mildewed (3) specimens is also disturbing. The failure of previous investigators to find viruses may well have been because there were no viruses, or it may be they did not see them because they were not looking for them. Shikita & Maramorosch (8) have emphasized the difficulty of finding virus even when it is known to be present.

Since ordinary TMV was maintained in the same greenhouse as these trials, a danger of contamination existed. Since most experimental isolates were strains of TMV, the confusion of experimental isolates with ordinary TMV was possible. Although some contaminations did occur, it is believed that they were not an important factor in this study. In addition to the argument that ordinary precautions were taken against contamination, the following points indicate that the infections reported probably came from the fungi tested: (i) It is hard to imagine how contaminating rods could be so consistently associated with certain infections, spores, and germ tubes as observed in the electron microscope, whereas other infections and spores were consistently free of rods. (ii) There is little opportunity for contamination when plants were sprayed or dusted with spores and the plants were not touched by human hands. (iii) Most of the infections were strains

of TMV, not previously seen, which protected against ordinary TMV in tobacco. (iv) The isolate from knotweed appears distinct from all other viruses observed.

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