long as the scale cannot turn edgewise (center, X, XII).

Irregular. Accretions with very irregular form usually reflect the shape of core on which they have formed. Fluted pieces of bedrock, and fragments of formations comprise the cores of most irregular accretions.

Associations of Rust and Virus Infections

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Attempts to transmit virus infections of plants by fungus and bacterial and nematode pathogens have generally been negative (*1, 2*), though the work of Hedges (*3*) may be an exception. Extracts of fungi usually inactivate viruses (*4*), no virus infection of fungi is clearly established, and no plant virus has increased with certainty *in vitro*. Therefore the greater susceptibility to several viruses of rust-infected than normal tissues may be of interest.

Interaction of viruses of tobacco mosaic (TMV), tobacco ring spot, tobacco necrosis, alfalfa mosaic, cucumber mosaic, white clover mosaic, beet mosaic, beet curly top, squash mosaic, and potato latent mosaic, with the uredinial stages of rusts of bean, sunflower, snapdragon, or beet have been tested. Positive evidence of association of the first five viruses with bean rust (*Uromyces phaseoli* on *Phaseolus vulgaris*), of the first two with sunflower rust (*Puccinia helianthi* on *Helianthus annuus*), and of the second with snapdragon rust (*Puccinia antirrhini* on *Antirrhinum majus*) has been obtained.

Bean plants about 8 days old and in the primary leaf stage were inoculated with rust race 1 by applying a suspension of uredospores with a brush to the lower surface of one half of each leaf and incubating overnight in a moist chamber. About 3 days later the leaves were inoculated with virus by applying with a firm brush a suspension of virus-infected tissue to the carborundum-dusted upper surface of one leaf of the pair of leaves on each plant. After inoculation the virus extract and carborundum were washed from the leaf surface.

On nonrusted tissues, virus symptoms usually appeared as noninvasive, necrotic local lesions in 3 days when tobacco mosaic, tobacco ring spot, tobacco necrosis, or alfalfa mosaic were inoculated on bean, but no specific local symptoms resulted from inoculation of cucumber mosaic on bean or of TMV or ring spot on sunflower leaves.

When TMV was inoculated on beans with well-separated rust pustules, necrotic rings formed around some of the pustules (Fig. 1). With closely contiguous rust pustules TMV infection formed few necrotic lesions, and did not form necrotic rings around the individual pustules. The virus was invasive in such rusted tissue, through which it moved about 1 mm per day, and formed a necrotic ring or margin around the entire rusted area. In virus-infected tissues, rust sporulation was reduced, and the tissue died sooner than in the absence of rust infection. The viruses of tobacco ring spot, tobacco necrosis, and alfalfa mosaic produced symptoms in rusted tissue somewhat like those of tobacco mosaic virus.

With cucumber mosaic virus on bean, circular necrotic lesions formed only in rusted tissues.

With TMV or tobacco ring spot virus on sunflower leaves no local lesions formed in the rusted or nonrusted areas, but assay of these tissues showed virus in both—more in the rusted than in the nonrusted areas.

Virus concentration in leaf tissues was measured by the local lesion method (*5*). In five trials the number of local lesions per square centimeter formed on *Nicotiana glutinosa* was 0, 0.02, and 0.08 for concentrations of 0.01, 0.1, and 1%, respectively, of tissues from TMV-inoculated nonrusted bean, and 0.3, 4.1, and 8.0 for 0.001, 0.01, and 0.1%, respectively, of tissues from TMV-inoculated rusted bean. For comparison the numbers were 2.2, 7.9, and 15 lesions for 0.0001, 0.001, and 0.01%, respectively, for tissues from tobacco systemically infected with TMV. When these data are plotted on log log scales, only the data for systemic TMV in tobacco gives the expected straight line, and it is therefore difficult to compare the virus concentration for these three types of tissues. If extrapolation of values is permitted, however, the writer believes, on the basis of the above

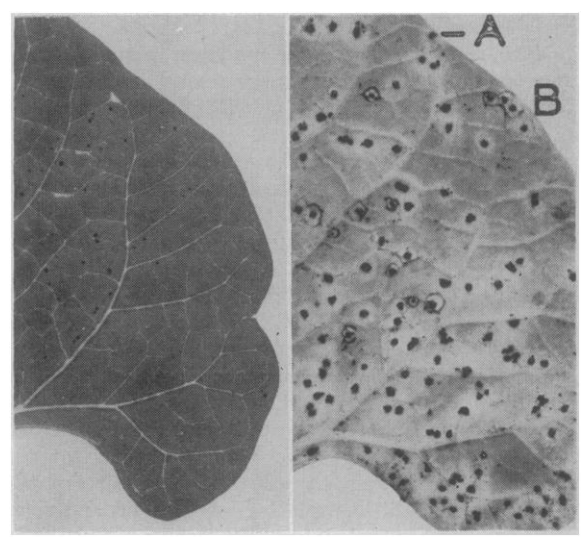

FIG. 1. Left, upper surface of pinto bean leaf showing ordinary local lesions caused by infection with tobacco mosaic virus. Right, lower surface of pinto bean leaf inoculated lightly with rust on May 12, and inoculated with tobacco mosaic virus on May 15; photographed May 31. Infection with tobacco mosaic virus appears as necrotic rings around some of the rust pustules: *A*, ordinary uredinial pustule without virus infection; *B*, a group of four uredinial lesions infected with virus. The virus infection apparently started in upper left pustule and has proceeded to lower right pustule, invading all contiguous pustules and forming progressively wider necrotic rings around the rust pustules. Similar symptoms were less distinctly seen on the upper leaf surface.

data, that the relative TMV concentration for TMV in nonrusted bean, rusted bean, and systemically infected tobacco was about 1 : 10,000 : 2,000,000, respectively. The value of 10,000 for the relative virus concentration in rusted in comparison with nonrusted tissue is not considered as finite or adequately determined. It has been shown to vary with age of leaf, age of virus infection, inoculum level of rust and virus, and bean variety.

If the virus concentration in the nonrusted tissue at about 15 days after inoculation is considered as 1, the virus concentration in other rusted tissues was as follows: 7 for tobacco mosaic in sunflower rust, 59 for alfalfa mosaic in bean rust, 440 for tobacco necrosis in bean rust, and 500 for tobacco ring spot in bean rust. With cucumber mosaic on bean, as assayed by local lesions on sugar beet, no such ratios can be indicated, for except for what is believed to be contamination in one test, none of this virus has been recovered from nonrusted tissue. Therefore the ratio of virus concentration in rusted tissue to that in nonrusted tissue was infinity. The bean variety Bountiful, of which nonrusted young and old leaves have been resistant to infection in the writer's tests, has been infected with TMV only when already infected with rust.

Although normal bean tissues become more resistant with age to TMV, tobacco necrosis, tobacco ring spot, and alfalfa mosaic, rusted tissues of similar age usually remain susceptible. Therefore it is likely that infinite differences in virus concentration between rusted and nonrusted tissues would result if beans of appropriate age were used.

With rust inoculation on sunflower plants already systemically infected with tobacco ring spot virus, no virus increase in association with the rust pustules was detected.

To measure the effect of rusted tissue on virus *in vitro,* fine suspensions of virus-free rusted and nonrusted bean tissues at different concentrations were added to TMV and tobacco necrosis virus at 0.01% tissue concentration. About 20 min after mixing, these suspensions were used as inocula on half-leaves of local lesion hosts (*N. glutinosa* for tobacco mosaic and *N. tabacum* for tobacco necrosis), and the virus suspension without supplement was used as inoculum on the opposite halves of the same leaves. At 0.003% concentration of rusted bean tissue, the number of TMV local lesions was 177% greater than for the control without rust extract, and this corresponds (from the straight line relating local lesions and virus concentration) to about an elevenfold increase in infective virus concentration. By the same token, a 0.01% concentration of bean rust tissue caused a five fold increase in infective tobacco necrosis virus. When these suspensions of virus and bean leaf tissues were allowed to stand overnight and then used as inocula, less stimulation of virus infectivity by rust tissues was detected.

Apparently significant smaller increases in infectivity were produced by nonrusted tissues at slightly higher tissue concentration. However, at 1% concentration, tissues from nonrusted and rusted leaves caused great reduction in virus infectivity, and the rusted tissue caused greater reduction than the healthy.

Although the above results must be considered preliminary, it is concluded that rust-infected plant tissues may increase the invasiveness and infectivity of certain plant viruses. No certain exceptions are known, and the several cases of negative results could be explained on the basis of inadequate trials, inoculation methods, or assay methods for the viruses used.

The cause of these associations has not been determined. That the greater susceptibility of rusted than nonrusted tissues to virus infection is not due to the mechanical punctures made in the cell walls by the rust haustoria is indicated by the finding that infection of bean with *Erysiphe polygoni* or *Colletotrichum lindemuthianum,* which also puncture the cell walls, has not been found to favor virus infection. The finding (unpublished) of Louis Jacobson, of the Division of Plant Nutrition, that rust infection increases the number and amount of free amino acids in bean leaves may have an important bearing on the results reported in this paper.

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Spectrophotometric Determinations of Esterases

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Salicylic acid strongly absorbs ultraviolet light of a wavelength of 290 mμ–300 mμ, whereas acetylsalicylic acid (aspirin) does not absorb at all in this region. This was found to hold true for other fatty acid esters of salicylic acid as well (Fig. 1). This principle provides a convenient and sensitive method for the determinations of esterases in general.

Because of the free carboxy group, these compounds, including the longer chain fatty acid esters, are soluble in solutions of low acidity and can be used as substrates in continuous spectrophotometric measurements. The hydrolysis of as little as 0.01 μM of such an ester can be detected.

The measurements were carried out in the Beckman spectrophotometer. The reference cell contained buffer, substrate, and water to a final volume of 3 ml. The control cell contained buffer, substrate, water, and enzyme to a final volume of 3 ml. The other cells contained, in addition to the elements of the control cell, substances the influence of which upon the en-