

BIG VEIN OF LETTUCE

A Virus Disease

Transmitted by

the Fungus

Olpidium

Brassicae

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Big Vein virus effects are visible on both lettuce leaves above, as compared with healthy leaf seen below.

BIG VEIN OF LETTUCE is a disease well known to lettuce growers in California and Arizona. The disease is characterized by coarse chlorotic bands along the leaf veins, as shown in photo, and by a delay in maturity and a reduction in head size. The disease does not kill lettuce plants and marketable crops are produced in spite of big vein infections. During the winter and spring when lettuce is shipped from the Imperial and Salinas valleys and big vein is severe, salad bowls across the country commonly contain lettuce leaves with big vein symptoms.

Big vein of lettuce was described in 1934 as a disease transmitted through the soil and possibly caused by a virus. In the succeeding 20 years, researchers assumed that it was caused by a virus, but its mode of transmission was not clarified. It had been reported to survive in air-dry soil for eight years. Attempts to transmit a virus from diseased to healthy plants failed except when roots of diseased plants were macerated and inoculated onto leaves of healthy plants. Later research showed that even this method of transmission failed if the macerated diseased roots were kept from reaching the roots of healthy plants. This report presents the results of research at Davis

during the last 10 years concerning big vein transmission.

In 1958 evidence was presented at Davis and in New Zealand that a microscopic fungus, *Olpidium brassicae* Wor., was intimately associated with big vein. A review of previous experiments with big vein showed no evidence that anything except *Olpidium* was responsible for transmitting big vein. There was also no evidence involving a virus because all attempts to demonstrate mechanical transmission had failed—or could be accounted for by contamination by the fungus. However, positive evidence was needed showing that either a virus transmitted by the *olpidium* caused big vein, or that big vein was caused by a toxic material produced by *Olpidium*.

The fungus

A study of the fungus shows that *Olpidium* forms a minute zoospore or swimming spore that is about 1/5000th inch across and propels itself through water by means of a long tail. In greenhouse tests, *Olpidium*-infected roots immersed in water will release as many as 200 million zoospores in a few minutes. Big vein is easily transmitted to healthy seedlings by placing a small quantity of

water with zoospores onto the root system. The zoospores come to rest on a plant root or root hair, infect, and develop into a single sporangium (spore sac) in the cell infected by the zoospore. The fungus does not produce hyphae and invade the root deeply as do some other fungi, such as *Fusarium* or *Rhizoctonia*. In three or four days these sporangia are mature and they release many zoospores. Thus, the life cycle is simple and short. The fungus also produces thick-walled resting spores that are capable of withstanding long periods of drying and chemical treatments. *Olpidium* has never been grown in the absence of a host plant and thus is an obligate parasite.

A method of grafting was developed in 1961 whereby crown tissue from *olpidium*-big vein-infected plants could be placed into crowns of healthy plants. The fungus was left behind in the infected roots, however, and only *Olpidium*-free tissue introduced into the healthy plant. When this was done, big vein developed in the healthy plants after four to five weeks. This was subsequently confirmed a short while later by another type of grafting experiment done independently in England. Up to seven consecutive graft transmissions of big vein were made with

Olpidium development on these plants. This provides ample evidence that a toxin produced by *Olpidium* is not involved in big vein but rather that an infectious agent multiplies in the lettuce, following transferral by grafting. Since no bacteria or other fungi can be discerned, we believe that this infectious agent is a virus and we refer to it as the lettuce big-vein virus, BVV.

Close association

The association between BVV transmission and *Olpidium* is close; however, the fungus is an obligate parasite and has only been grown in living plants. As a result, the zoospore suspensions that are used to transmit BVV contain bacteria, and many flagellates other than *Olpidium*. Several lines of evidence indicated that *Olpidium* was functioning as the vector: soil transmission was lost when big vein was transmitted by graft-inoculation and thereby freed of *Olpidium*; removal of *Olpidium* from zoospore suspensions by filtration, heat treatment or dilution prevented BVV transmission; BVV-free *Olpidium* isolates acquired BVV from roots of grafted plants; *Olpidium* and BVV survived air drying infected roots.

Although the big-vein virus can be transmitted by grafting, as well as by *Olpidium*, all other methods that have been tried to date have not succeeded in transmitting BVV. Thus, it has been impossible to grind up infected leaves and to isolate BVV and to demonstrate by inoculating lettuce plants that the isolated virus causes big vein. Analysis of healthy and diseased plants has not given any consistent differences that would indicate the nature of the virus.

The *Olpidium* zoospores apparently carry the virus internally, because repeated washings do not remove the virus; however, there is still a possibility that the virus may be tightly stuck onto the outside of the zoospore. A BVV-carrying isolate of *Olpidium* was freed of the virus after several consecutive transfers on sugar beet. Apparently sugar beet is a host for the fungus but not for BVV. The fact that BVV survives for the first few transfers on sugar beet suggests that it is persistent in the *Olpidium*, although it is possible that the virus multiplies very slowly in sugar beet as compared to lettuce. Some BVV-*Olpidium* isolates have been obtained by heating the fungus enough to kill most of the zoospores or by diluting the zoospores so only one or a few infect. It is not clear in these cases whether BVV was actually inactivated, whether the surviving zoospores hap-

pened to be free of BVV before the experiment began, or whether such a small amount of BVV was introduced to the host that it was not able to infect successfully. At any rate, the combination between the virus and the fungus is not easily disrupted and the most probable explanation seems to be that the zoospores carry the virus internally.

When roots of *Olpidium*-infected plants were air-dried for up to five months, then thoroughly macerated and sieved through fine sieves, the resting spores were still intact and had passed through the sieves. Lettuce seedlings were inoculated with these untreated resting spores as well as spores treated with either a strong acid or strong alkali. These treatments destroy most viruses and the acidification destroyed the resting spores in some experiments.

Whenever *Olpidium* survived the chemical treatments, however, it transmitted BVV as well as the untreated controls. It seems most likely that the virus survived these treatments, and the air drying, because it was within the resting spores of *olpidium*. This would account for the reported survival of big vein in soil that was kept air-dry for eight years. A few of the number of virus diseases known to be soil-borne, have the ability to withstand drying of the soil and yet infect seedlings when they germinate in this previously dried soil. These include lettuce big vein, tobacco stunt virus, and soil-borne viruses of oats, wheat and barley. It seems probable that these viruses survive within their vectors and that their vectors are similar to *Olpidium*. It was suggested in 1960 experiments that the behavior of tobacco stunt virus correlated with the behavior of *Olpidium*.

As was previously mentioned, BVV-free *Olpidium* has been obtained by experimental treatments of BVV-carrying *Olpidium*. An easier method of getting BVV-free *Olpidium* is to isolate this fungus from lettuce or from a number of other hosts in areas where big vein does not occur. There is some evidence that other hosts of the fungus are infected by BVV-carrying *Olpidium* in the lettuce-growing areas, such as the Salinas Valley. It has been reported in England that a BVV-free isolate of the fungus could acquire BVV from the roots of grafted plants when incubated on them for two weeks.

In California, two BVV-free isolates—one that had BVV and was freed of it by transfer on sugar beet, and one that never had BVV—were inoculated onto grafted

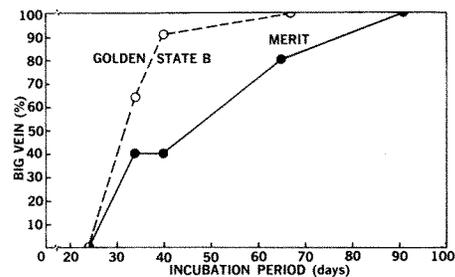
plants. The first generation of zoospores formed on these grafted plants was recovered and shown to transmit BVV. Thus a BVV-transmitting isolate of *Olpidium* can be freed of BVV and then can reacquire BVV. Once BVV was reacquired, the fungus continued to transmit it.

Control

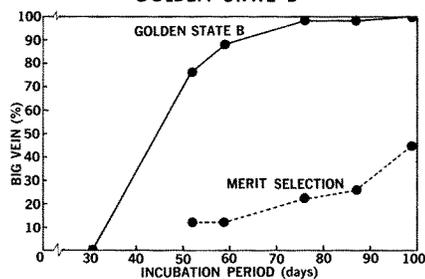
There are two approaches to control of big vein. The first involves chemical treatments aimed at killing the fungus, *Olpidium*, or preventing germination of resting spores during the early part of the growing season. This would prevent transmission to young plants that are most seriously set back by big vein. Some success with soil applications of PCNB (Pentachloronitrobenzene) and chloropicrin have been reported from Arizona. Experiments are under way in Monterey County, to determine if a feasible treatment can be developed. For lasting protection the nature of *Olpidium* requires either that chemical treatment completely eliminate the fungus from the soil or that a chemical with a long residual action be used to suppress infections. Either objective is difficult to achieve in the field and it will probably require several years testing to work out satisfactory chemical control.

The second possibility of control is to breed lettuce varieties resistant to BVV. Big vein resistance of some recently released varieties has been evaluated at Salinas. Merit and Caravan were found moderately resistant and Calmar was tolerant. In some plots the resistant lines developed about as much big vein as the susceptible checks. During the research on big-vein transmission by the fungus, techniques were developed for early and severe inoculations of seedlings with big vein virus by using large numbers of *Olpidium* zoospores. These techniques have been applied in the program of breeding for big vein resistance and have made it possible to screen out the most resistant plants from a population and to minimize the possibilities of any plants escaping infection. With these techniques,

BIG VEIN TOLERANCE OF MERIT COMPARED WITH A SUSCEPTIBLE VARIETY, GOLDEN STATE B



BIG VEIN TOLERANCE OF MERIT SELECTION
COMPARED WITH A SUSCEPTIBLE VARIETY,
GOLDEN STATE B



20 of 20 plants of a susceptible variety had big vein within 67 days of inoculation, whereas Merit had 20 of 20 plants with big vein in 91 days. This indicates that BVV resistance of Merit can be overcome and is one reason why Merit might have as severe big vein as susceptible varieties in some fields. However, when the progeny of the most resistant plants from this screening were tested the following year, they had only 45% big vein in 99 days compared to nearly 100% big vein in the susceptible checks at 76 days. Apparently a rigorous screening and selection program can greatly improve the big-vein resistance of Merit. This possibility is being tested now using a susceptible commercially grown variety.

A Merit plant, selected for resistance, was crossed with Great Lakes A-36 to incorporate big-vein resistance with the Great Lakes horticultural characteristics. The F₂ and F₃ progeny were screened by severe inoculations in the greenhouse and the most resistant plants selected. These will be screened again in the greenhouse for resistance and also planted into the field to determine their horticultural characteristics. The development of big-vein-resistant varieties appears especially promising at the present time.

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Recently Developed Vegetables Aid Mechanization and Climate

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A CONSTANT SEARCH is carried on by scientists of the Department of Vegetable Crops at Davis and Riverside for plant breeding materials—among both wild species and cultivated varieties—that will contribute resistance to specific plant diseases of concern to the vegetable industry. After crosses are made between the resistant types and commonly cultivated varieties, a prolonged process of backcrossing and selection has to be followed. The progenies have to be checked and rechecked to be sure that the resistance is not lost. Ultimately the breeding lines are brought back to horticulturally desirable types. Then they have to be tested for their adaptability to the various climatic areas of the state—usually with the help of county farm advisors. The processing and shipping ability of the crop has to be determined, as well as edible quality. Only after all of these evaluations is the decision made to release a new variety to the seed industry for seed increase and distribution to growers. This report reviews some of the varieties developed in the past few years that have found a place in the state's production, as well as some new varieties just released.

Tomatoes

Imperial, released in 1959, is a market-type tomato with resistance to Fusarium wilt. It was developed in the Nyland district of the Imperial Valley and is adapted primarily as a fall shipping tomato for that area. The plants are determinate, light green in color and produce a good cover. The fruits are a flattened globe shape, of good size and with a tendency to have an open style.

VF36, released in 1959, is resistant to Fusarium and Verticillium and produces a tomato suitable for canning. It is two weeks earlier than Pearson and is one of the earliest resistant strains suitable for

processing. Yield trials indicate that it is equal to other resistant varieties. Canning tests reveal that it is equal or superior in quality to other leading varieties. The vine size is smaller and more open than Pearson. Fruit size has been consistently as large and the fruits as firm as Pearson.

(The prefix VF has become standard to denote resistance to Verticillium and Fusarium wilts.)

VF14, released in 1961, is also resistant to Fusarium and Verticillium and is suitable for canning. It is a week later than VF36 and is, therefore, a second early resistant variety. It has set fruit abundantly during the high temperatures that prevail in the Sacramento-San Joaquin valleys of California. This ability to set at high temperatures and the time of maturity make it a good companion variety to be planted with VF36.

VF14 also carries the uniform ripening gene. Fruit is oblate in shape with some tendency for navel formation at the blossom end on fruit of the first clusters. Soluble solids and firmness of fruit are similar to VF36 but the pH is somewhat higher. The vine size is about the same

