ANALYSIS OF THE VIRUS DISEASE COMPLEX THAT REDUCES YIELDS OF BLUE LAKE BEANS IN OREGON

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SUMMARY

Project began by making systematic surveys of Blue Lake beans in Oregon plantings. This survey did not depend on merely looking at the plants, but was accomplished by collecting representative specimens and analyzing them with extensive test-plant layouts and with good greenhouse facilities. The survey indicates:

1. While most of the plants having mottles considered to be of genetic origin tested virus-free, a few tested virus-infected. An obscure virus complex may thus obtain in some of the off-type chlorotic patterns previously attributed to genetic factors alone.

2. Both field observations and subsequent test plant analyses indicate that bean common mosaic was not present in the Blue Lake plantings. On the contrary, a Blue Lake malady mistakenly attributed to that virus by others, proved to be due to western ringspot virus, an entirely different virus.

3. Bean yellow mosaic continued to be generally present in all the areas Blue Lake beans are grown. An unusually severe strain of that virus was present in some fields in the Portland area.

4. The name, "sudden death disease," is assigned to a quick-wilt of large bean plants due to a little known virus, western ringspot. This disease was very noticeable in the Grants Pass area and occurred also in the Willamette Valley. Extensive studies of the causal virus are being made.

5. Further immediate investigations should emphasize (a) critical testing for viruses in genetic complexes, (b) finding sources of the severe bean yellow mosaic strains in the Portland area and, (c) finding the source of western ringspot virus in other areas. Should "sudden death disease" become general, the bean growers would face a catastrophe.

OBJECTIVES

Principal aims of this project are:

1. To obtain basic information about viruses recurrently present in Blue Lake beans to avoid catastrophes such as happened to FM-65.

2. To determine whether some obscure infectious virus may predetermine the occurrence of economic losses from root rot in Blue Lake beans. Serious root rot recurs spasmodically in a manner that indicates some factor other than the obviously associated fungi may enable the disease occasionally to limit production.

It was thought that the project should begin with general field surveys specifically planned to:

1. Prove or disprove that the "genetic mottles and growth abberrations" present in all strains of Blue Lakes are truly chromosome phenomena and not partly caused by undetected viruses.

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2. Describe the virus-like maladies currently present in the Oregon Blue Lake plantings and determine the viruses responsible.

METHODS

Technical phases of this study were handled by the authors.

For this project, at least 700 plants were made available each week by planting seeds in cans every 7 days. These were grown in a greenhouse where they could be protected from contamination by insects. Also, a separate greenhouse was ground-planted to Blue Lake varieties to provide test plants under conditions where growth of the plants simulated growth of beans in field plantings. These plant materials sufficed to prove which viruses were responsible for certain conditions observed in bean fields.

Standard procedure was to inoculate from the Blue Lake bean plant in question to four kinds of phaseolus bean--Top Crop, Bountiful, Dwarf Horticultural, and the FM-1 variety of Blue Lake--and to Vicia faba, the broad or horsebean. Previous experience had shown these test plants were advantageous and this season's results justified their choice. Use of these kinds of test plants should detect those viruses naturally present in Blue Lake beans. To identify certain of the viruses isolated, 10 other plant species, in addition to these test plants, were planted and maintained as needed.

EXPLANATION OF VIRUS TERMS

Genetic Mottles: Certain variegations and various bright colored mottles, such as those of coleus, are often related to the genetic constitution of the plant that varies from seedling to seedling. Unfortunately, certain viruses also produce similar color patterns easily confused with genetic mottles. The term genetic mottle, as used in this report, means the experimenters considered the abnormal colorations more likely due to genetic variations associated with the mechanism of fertilization and seed formation than to viruses transmitted in the seed or introduced during plant growth.

Bean Common Mosaic Virus: The cosmopolitan seedborne virus of Phaseolus beans usually apparent as a coarse green mottle (figure 7). This virus was one of those responsible for the former losses of FM-65 plantings in Oregon and Washington. Currently used Blue Lake varieties are highly resistant to it. It has a narrow host range infecting only a few species of legume plants. Former investigations indicated, however, that there was an unknown source or "reservoir" for this virus in the Pacific Northwest.

Bean Yellow Mosaic Virus: Another cosmopolitan virus widely distributed in the legume family. This virus differs from bean common mosaic by (1) not being seedborne, (2) having a tendency to produce yellow, not green mottles, (3) having a wide host range that includes gladiolus and other bulb type plants that belong to the Iris family, (4) forming countless strains that are distinct with respect to (a) host preference for different bean varieties and legume species and (b) ability to injure or kill Blue Lake beans, (5) being known to overwinter in various perennial plants, especially clovers.

Western Ringspot Virus: A recently discovered and comparatively unknown virus that is able to quickly kill Blue Lake beans just as they reach the harvest stage.

This season's investigations have proved that western ringspot virus is responsible for the sudden death disease of beans formerly attributed to bean common mosaic virus.

Enation Mosaic Virus: This is the virus that yearly renders thousands of acres of peas unmarketable. The virus is not seedborne but it does live in aphids, thus accomplishing spread over such distances as aphids are carried by winds. Causes tiny growths called, "enations" to form on leaves and clear hyalin windows in leaves. Usually found in garden and field peas, in vetches, and in crimson and subelover but recently proved by us to be able to infect Phaseolus beans.

GENETIC MOTTLES IN BLUE LAKE STRAINS

Leaf variegations mottles, and various chlorophyll deficiencies have long been known to occur in beans, and have been studied by various investigators. Some have been shown to be transmitted by the cytoplasm not the chromosomes; others through the mechanism of recessive or dominant genes contained in the chromosomes. Some "genetic mottling" was noted in Oregon Blue Lakes in 1954, but an alarming amount was noted in 1955. It is not known whether this increase relates to germ plasm factors alone, to conditioning by the unusually cool weather, or to a combination of the two. Some abnormalities may also have been caused by viruses. The problem has thus to be considered both from virus and genetic viewpoints. For the latter, seeds from selected abnormal plants were saved this season to determine the nature and mechanism of transmission.

Between early July and late August 12 samples of abnormalities apparently of genetic origin were collected and analyzed as indicated in table 1. The data in this table include abnormalities observed in various strains of Blue Lake beans regularly grown.

This test indicates that recognition of abnormalities due to genetic abberrations alone is more difficult than anticipated. Collection 197 for example, rated absolutely typical bright yellow genetic symptoms, certainly contained a high

Table 1. Collections of Foliar Abnormalities Considered to be of Genetic Origin

Col.	Area	Bount	DwarfHort	TopCrop	Broadbean	FM - 1
152	F.Grove	no	-	-	no	-
153	Eugene	no	-	-	?	no
154	Eugene	-	-	yes	-	no
155	Eugene	-	-	no	-	no
160	St.Paul	no	no	no	no	-
177	Eugene	yes	no	no	no	yes
178	Eugene	no	no	no	no	no
181	Eugene	no	no	no	no	no
197	G.Pass	yes	yes	yes	yes	yes
223	V.Buren	no	no	yes	no	no
224	V.Buren	no	no	no	no	no
225	V.Buren	no	no	no	no	no

Legend: "yes" means test plants indicated presence of a virus; " - " means the variety of bean was not used because plants were not available when the test was made.

concentration of bean yellow mosaic virus! In that case it may be presumed that the genetic-like mottle plant was attacked also by our most common virus. Subsequent tests indicated that the appearance of the Blue Lake plant in the field could not be explained by the presence of that virus alone. Thus in 4 out of 12 collections tested, the presence of some virus was indicated, and 1 of the 4 was certainly bean yellow mosaic. The occurrence is so stated to emphasize the circumstance that the virus or viruses detected in the other 3 collections were obscure, their apparent transfer was low and erratic with respect to host preference, and they were not identified during the course of this investigation. That is to say, the reactions of some test plants indicated a strange virus was present in the Blue Lake sources but performance of the test plants failed to place or name the viruses detected. From this experience we can plan a test next season to distinguish more adequately the border between genetic troubles and virus troubles in Blue Lake beans. This test should consider the possibility that not only chromosome units but also cytoplasmic units (plasmagenes) are concerned in the genetic mottles. The plasmagene suggestion is enticing because there may be a relation between plasmagenes and viruses.

The genetic-like bright mottles were not due to genetic effects alone when the veins on the leaves appeared burned. Strains of bean yellow mosaic were consistently isolated from such leaves and this atypical condition is not considered in the above discussion of genetic mottles.

Summary of the genetic mottle discussion: More indications of virus presence were found in the "genetic mottles" than were anticipated. It is possible that some unknown virus may occur in the genetic complex.

VIRUS DISEASES OBSERVED

A. Symptom types in bean plants. Field observations and conferences with fieldmen indicate there was less loss from virus diseases in Oregon's Blue Lake plantings this year than ever before. Economic loss from virus diseases was apparent only in certain fields in the Columbia Highway area. One reason is the unexplained fact that the fields were not invaded by viruses early in the 1955 season. During an average year, early-season injury from virus is noted by dwarfed plants that bear tiny yellow mottled leaves and form no beans. These plants are usually abundant in the outer rows where they are especially exposed to virus brought in from neighboring crops and weeds by migrating insects. These plants, in turn, can serve as new sources of virus that lead to general spread within the planting. Those circumstances that prevented early introduction of virus may account for the comparatively small amount of virus disease this year.

Virus symptoms vary with species of virus, virus strain, species of plant, variety of plant, age of plant when infected, prevailing temperatures, and many other circumstances. Detailed interpretation and record of symptoms observed in Blue Lake beans this season are, therefore, beyond the scope of this report. A short discussion is given of seasonal occurrence and regional distribution of symptom types to orient the presentation of data about those viruses and diseases considered economically important.

Several deficiency diseases, especially potassium deficiency, were very noticeable early this season. Samples of these were sometimes sent in for virus determination; of course no virus was found. An unusual early season trouble, apparently of virus origin, was severe dwarfing without the usual yellow mottle. Such plants were observed only in the St. Paul vicinity, a comparatively new bean area. Principal cause of this dwarfing was not determined.

No symptoms of bean common mosaic were observed in any of the fields, and no tests indicated presence of that virus.

Characteristic effects from virus invading late season are shown in figure 2 representing a row in a field severely damaged by virus. Symptoms indicating presence of virus diseases in Blue Lake beans so infected, vary from mild yellow and green mottles to drying of leaves, blackening of leaves, and death of plants. Those symptoms that herald death begin as necroses that vary in color from brown to black. No typically virus diseased plant yielded any marketable beans. More than 100 plants having various symptoms were sampled by the test plant methods mentioned above. From this sampling, the following distribution of viruses for the area was deduced.

In all areas some mottled or mildly necrotic plants were found that were diseased with bean yellow mosaic, and usually with that virus alone. That was expected because that virus is certainly present in every county in western Oregon. Initial sampling from the Forest Grove area indicated that occasional severe blighting of plants in that area was due to yellow mosaic combined with a second virus that was not identified this year. Forest Grove plantings should be surveyed again with plant materials specifically chosen to isolate and identify the second virus.

Black necrotic blighting of the terminal parts followed by sudden death of vines was general in the Grants Pass area and rare in the Eugene area. This condition proved to be a specific disease due to western ringspot virus. The name "sudden death disease" was chosen to designate this disease distinctly from other bean troubles.

Three different types of virus diseases were identified in the area this year, namely, the "sudden death disease" and two distinct forms of bean yellow mosaic. Every bean plant infected with one of these becomes a complete economic loss. That emphasizes the need for specific knowledge about the causal viruses to prevent their becoming general. Field observations and test plants also indicated the presence of two other viruses that remain unidentified.

B. The bean yellow mosaic problem. Bean yellow mosaic is the most common virus disease of beans in the Pacific Northwest and in the northern part of the United States from coast to coast. The virus that causes bean yellow mosaic invades most of the principal legume crops grown in this country, and recently has become generally present in Europe. There is great international interest in this virus.

Bitter experience and related emergency investigations have taught many facts about bean yellow mosaic virus as a frequent invader of Blue Lake beans. Thus experienced canners' fieldmen avoid planting Blue Lakes near old red clover sod or gladiolus fields. The disease, however, continues to be a hazard for canners concerned with beans and peas, and further specific information about the virus is needed.

Whether viruses should be placed among the quick or the dead is a question of academic import, but their chief claim for living status--their ability to mutate--is truly a circumstance of economic importance. No virus surpasses bean yellow mosaic with respect to that ability. Thus there are countless strains of this virus that have love-for-beans as a common characteristic, but vary unbelievably with respect to effects on the plants. Conversely, as has been shown by one of our students (James Baggett), genetic changes in Blue Lake beans result in profound response variations to the same strain of the virus. A reliable method for classifying strains of this virus is greatly needed, and is one of the current research objectives at the Oregon Experiment Station where breeding resistance into Blue Lake beans is being attempted.

During the course of this season's survey many isolations of this virus were made. A significant finding in this investigation is that when the isolation-ability of the test plants used for isolations is plotted against the geographic area where the virus was collected or against the type of symptom, there is specific grouping of the test plants. Typical yellow mosaic from fields south of Salem gave the expected performance in the test plants; collections from the severe form of the disease observed in Portland and Columbia Highway areas gave the unusual vein symptoms shown in figure 3. Further analyses of symptoms observed in the field and of test plant reactions, indicate that an unusual group of strains are occurring in that region. Thus, in the northern Oregon area the strains regularly produce both classical mottle and also necrosis in the beans. Those in the other sections of the state remain of classical type. Recognition of such divergent strains is important to those trying to breed resistance to this virus in Blue Lake lines.

Complete cytological studies and limited host range investigations are being made of the strains of bean yellow mosaic collected during the course of this bean survey.

C. Top wilt or "sudden death disease". The name "sudden death disease" indicates a serious disease specifically due to a little known virus provisionally christened western ringspot virus. While the progress of pathogenesis varies with strain of the virus and variety of bean, the production of intensely black dead parts followed by the collapse of the plant are symptoms that always accompany the presence of this virus in Blue Lake beans. Before this general necrosis occurs, small star-shaped lesions (figure 5) may form on some leaves; then the latest formed trifoliate leaf crinkles and has the appearance of a poison oak leaflet (figure 6). These symptoms are followed by an amazingly rapid black necrosis that can cause the complete collapse of a 6-foot plant in less than 5 days.

Neither the virus nor the disease is new. Apparently, the same disease was previously noted in Lane County and there termed bean common mosaic (BCM) by visiting experts, because it was shown in the East that BCM-virus can produce such symptoms in a bean variety resistant to BCM-virus. When dealing with virus diseases, the old axiom "all that glitters is not gold" is especially apt. This observation is made to emphasize that only through such an approach as this project permits can one avoid mistakes in diagnosis that in time can become serious. In the present instance we know that this virus, whose effects were noted as a curiosity a few years ago, is now general in Oregon and directly related to several obscure troubles in canning crops.

One distressing feature about the disease, as it occurs in Blue Lake beans, is that it never becomes apparent until plants climb nearly to their final height. The habit of developing late is distressing. Were the disease to become general the crop would be a total loss, although the disease would not be detected until the time of first picking. A plausible explanation of this late invasion was found by studying the infectivity of several strains of the virus to Blue Lake beans of different ages. While direct transfer of Blue Lake to Blue Lake gave a good initial transfer, subsequent transfer to seedling Blue Lakes from other kinds of plants, for example broadbeans, was very difficult. In fact several trials with the five isolates or strains of the virus transferred from heavily infected broadbeans to seedling Blue Lake plants were entirely negative. That observation suggested that the disease in the field might be due to a virus complex of which the western ringspot virus was only one component. Later, these same strains again transferred from broadbean, were tested on Blue Lake plants 2 to 3 feet tall. Each strain transferred readily to these older beans and perfectly reproduced the symptoms originally observed in the field. That is to say, the older Blue Lake beans are more susceptible than the young when the virus is being moved from some other kind of plant to Blue Lake beans.

The presence of this virus in Blue Lake beans is indicated by the above symptoms, and proved by isolating it into broadbean then subtransferring into tobacco species where it produces characteristic symptoms. Thus, it has certain factors in common with the virus that causes alfalfa mosaic, also, certain resemblances to the classical ringspot viruses. Alfalfa mosaic virus often produces coarse mosaic mottles in bush beans; classical ringspot viruses produce mosaic mottles in cucumbers. This new virus, has proved to be WRS, a killer virus in susceptible leguminous plants, with the possible exception of sweet clovers, and does not usually transfer to cucumbers. Thus, it is not a mosaic virus like the other viruses usually found in Blue Lake beans but is an entity distinct from the other viruses that include leguminous plants in their host ranges.

Western ringspot virus, WRS, often accompanies other viruses in plants and, when so present, has a little understood relationship to the diseases of several agricultural crops. Table 2 shows the geographical areas where it has been found and the other viruses present in the sick plants from which the WRS virus was collected. Only in dying Blue Lake beans has this virus been found occurring alone. Investigations of pea diseases in Eastern Oregon have shown that this virus combines with enation mosaic virus, and produces a severe wilt of peas. It has been isolated from potatoes, but its relation to potato diseases is not known. In Blue Lake beans it sometimes occurs along with bean yellow mosaic in plants having yellow mosaic symptoms. Strange to relate, the combination disease in Blue Lakes is much less severe than that induced by Western ringspot alone. When present in natural infections with yellow mosaic virus, the WRS-virus is present in very low concentrations. The demonstration that this virus alone is the killer-virus in fields of Blue Lake beans, was not anticipated. With respect to the pea situation where terminal wilts are becoming more and more important, a study of the insect transmission of this virus was made by Mr. Amen. He showed that the pea aphid when offered this virus alone is unable to retain and transmit it with efficiency. On the contrary if the virus is mixed with enation mosaic virus the aphid becomes an efficient carrier of the WRS-virus. Such specific findings make it difficult to explain how the virus enters bean fields late in the season.

Table 2. Summary of Western Ringspot Collections

Location	Crop	Other Virus
Milton.....	Peas	Enation Mosaic
Ontario.....	Potato	Alfalfa Mosaic
Stayton.....	Blue Lake	Bean Yellow Mosaic
Eugene	Blue Lake	(none)
Grants Pass.....	Blue Lake	(none)

Table 3. Relative Efficiency of Different Test Plants for Isolating Strains of Western Ringspot Virus from Blue Lake Beans

Col.	Area	Bount	DwarfHort	TopCrop	Broadbean	FM - 1
179..	Eugene	Yes	Yes	Yes	Yes	Yes
201..	G.Pass	Yes	No	Yes	Yes	Yes
203..	G.Pass	No	No	No	Yes	No
204..	G.Pass	Yes	Yes	Yes	Yes	Yes
205..	G.Pass	Yes	Yes	No	Yes	No

The importance of this virus, measured by its ability to destroy bean and pea crops, were it generally introduced, warrants thorough study. With cooperation of the Department of Biochemistry, we are making a study of the virus itself to obtain data for placing it among other members of the virus family. Following preliminary investigation last summer by Leroy Kuehn and Dr. Tsoo King, sponsored by a National Science Foundation grant, biochemical studies are being conducted. Progress has been made toward purifying the virus so that it can be characterized at the molecular level. From purified virus preparations its physiochemical constants can be determined, the mechanism of low or high infectivity and synergistic action with other viruses investigated, and its serological behavior studied. These paths are being followed with the recent techniques of chromatography, counter-current extraction, ionophoresis, and other methods applicable to the study of viruses.

Electron microscopy practiced during the course of these physiochemical studies is already placing this virus close to the true ringspot viruses. These studies are mentioned to emphasize that we consider this a comparatively new and exceptionally important virus whose relationship to the Blue Lake beans and other crops must be determined.

As mentioned above, this WRS-virus sometimes occurs with bean yellow mosaic. This suggests that it could be a strain of that virus. That supposition was completely disproved by a series of protection tests using strains of western ringspot currently collected from Blue Lake beans as a challenger virus against bean yellow mosaic virus established in broadbeans. The BYM virus produces a mild mosaic disease in broadbean, and the plants survive and grow tall. Not only did the yellow mosaic fail to protect against the western ringspot virus but the WRS-virus quickly killed the mosaic diseased broadbean plants! Protection tests are comparable to immunological studies and are considered the best means for relating viruses. The western ringspot virus is unrelated to bean yellow mosaic virus.

Specifically for this Blue Lake project, we are making extensive host preference and host range studies. This procedure is necessary for organizing a search for the source of the virus. When we demonstrate a plausible source we can look for the carrier, insect or otherwise, and begin tracking the virus from its source to Blue Lake beans. In the above outline of studies of the virus it was stated that current findings are placing the virus near true ringspots. Translating that observation into practical control thinking we know that some mode of spread other than by insects may be the principal mode of entry. Thus, a general study of this virus is being made as a contribution to a serious problem that may face the growers and processors of Blue Lake beans.

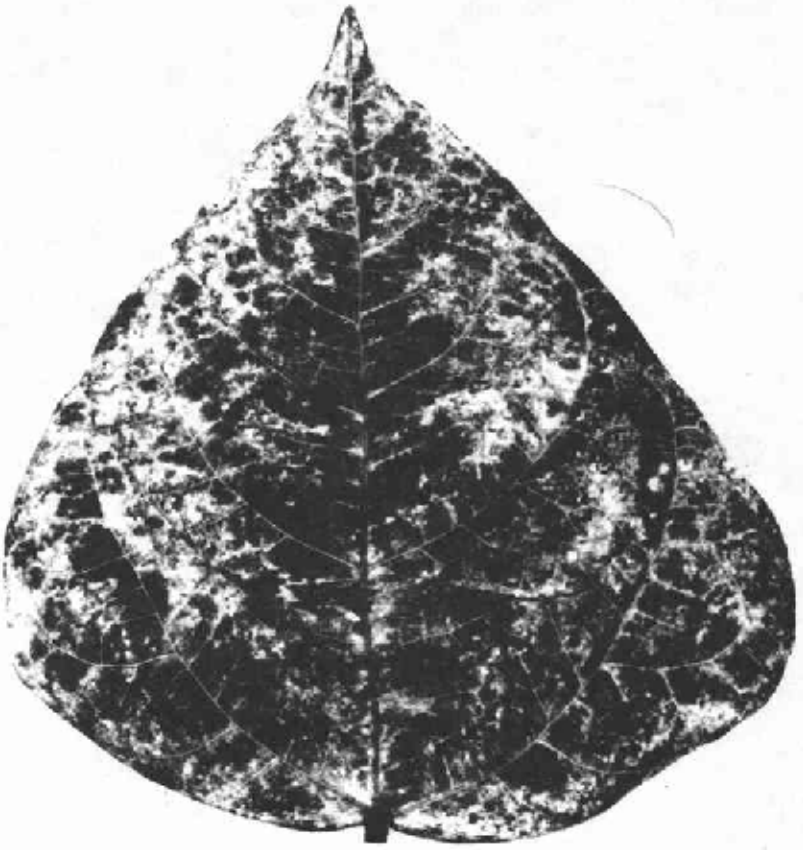

Figure 1. Bright or genetic mottle in Blue Lake beans.

Figure 2. Bean yellow mosaic in Blue Lake beans. Large leaves are on healthy plants; small, curled leaves on diseased plants.

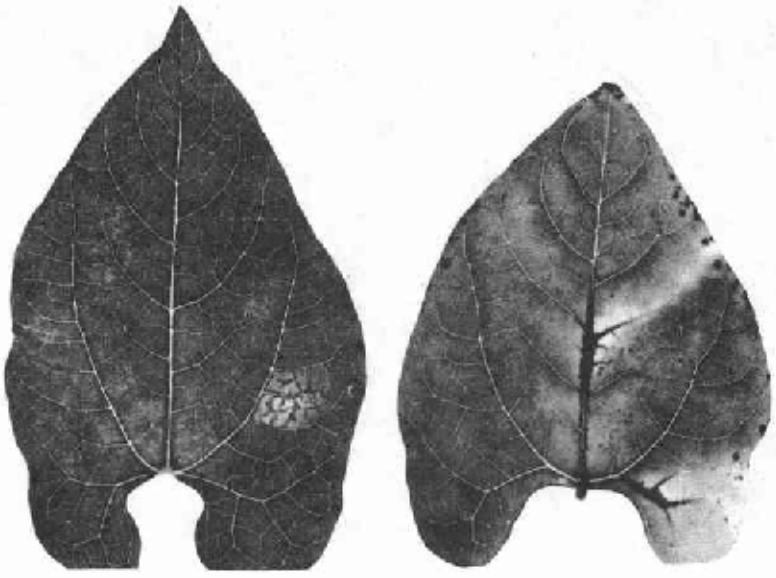

Figure 3. Purple vein patch in Top Crop.

Black vein in FM-1 247.

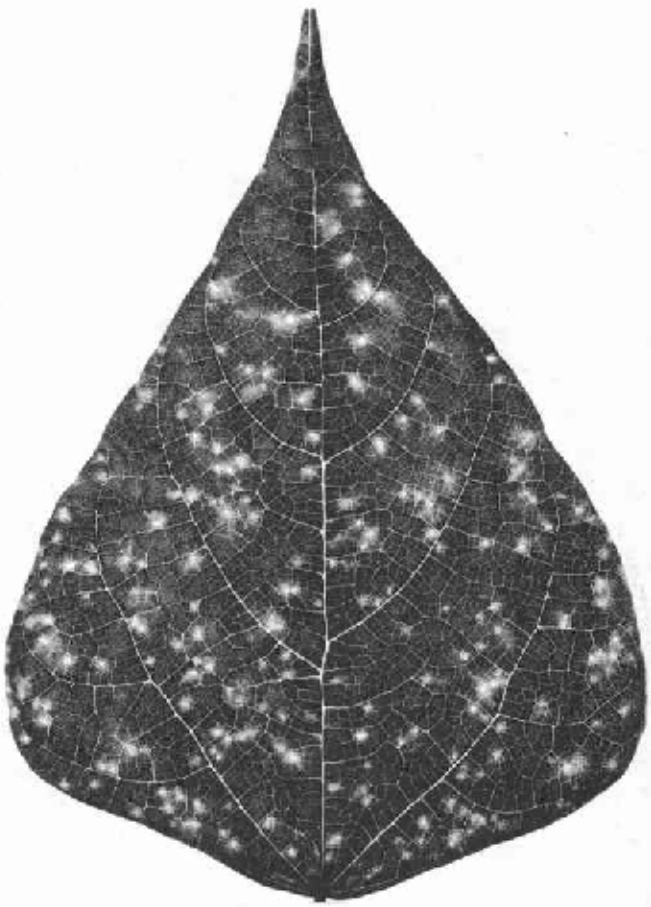

Figure 4. Systemic symptoms of severe strain of yellow mosaic in Bountiful beans.

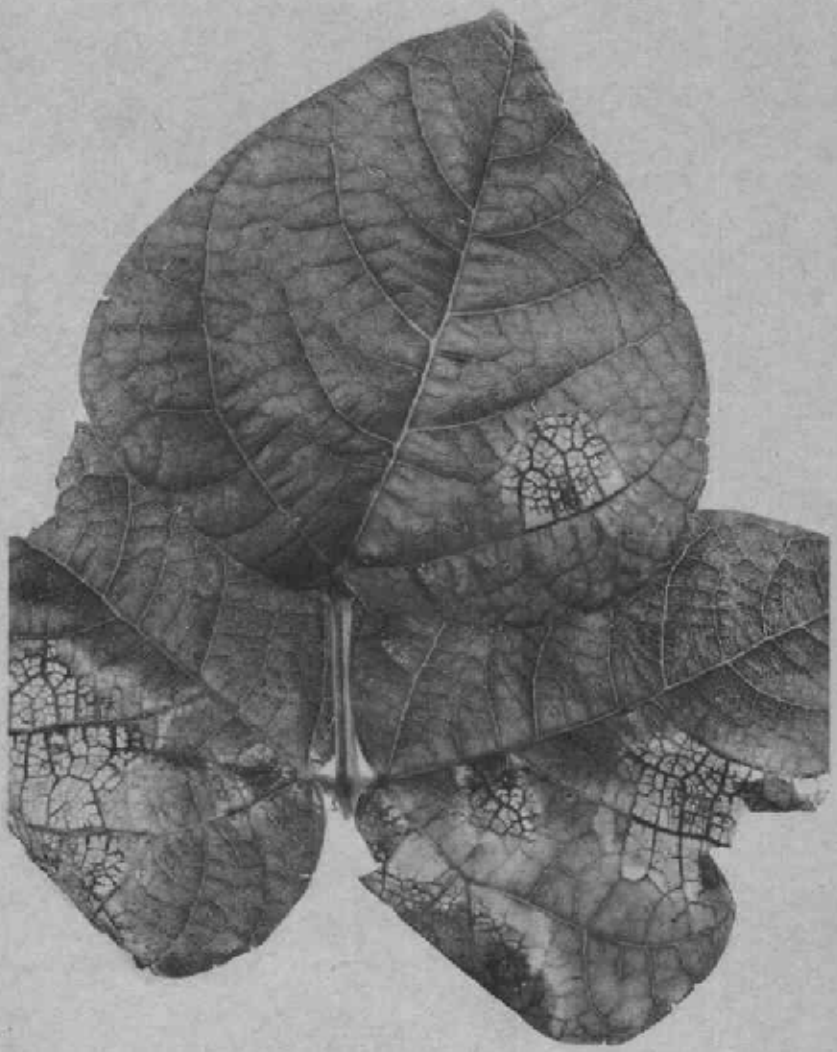

Figure 5. Primary systemic symptoms of Western ringspot virus that causes sudden death disease of Blue Lake beans.

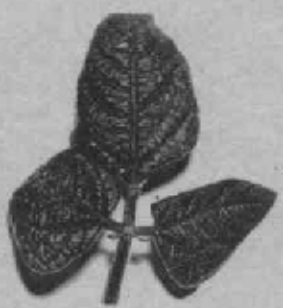

Figure 6. Leaflet showing the "poison oak symptoms" of sudden death disease in Blue Lake beans.

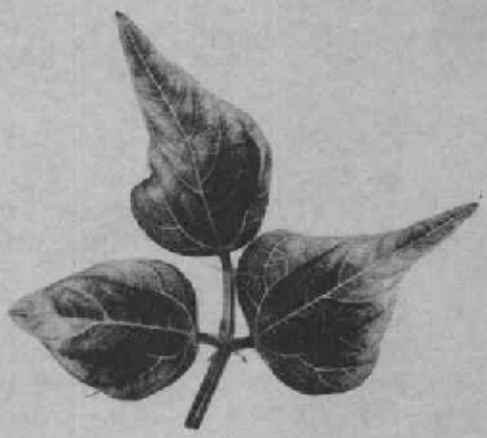

Figure 7. Common bean mosaic in Dwarf Horticultural beans.