# EVALUATION AND INHERITANCE OF RESISTANCE IN FABA BEAN (*Vicia faba*) TO BEAN YELLOW MOSAIC VIRUS

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Inder Paul Singh Gadh

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# FOREWORD

The format adopted for this thesis deviates from the conventional and has been approved by the Council of the Faculty of Graduate Studies at the University of Manitoba. Accordingly, materials, methods and results are presented in the form of three publications, the style of which complies with the requirements of the Canadian Journal of Plant Pathology. A general discussion follows and thesis terminates with bibliography and appendices.

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## GENERAL ABSTRACT

Gadh, Inder Paul Singh. Ph.D., The University of Manitoba, May, 1982. Evaluation and Inheritance of Resistance in Faba Bean (Vicia faba) to Bean Yellow Mosaic Virus. Major Professor; Dr. C.C. Bernier.

Sixty-eight open-pollinated faba bean selections identified as resistant to bean yellow mosaic virus (BYMV) in preliminary field experiments were evaluated further against the 'mosaic' strain of BYMV in the field from 1978 to 1981. Most selections were variable for disease reaction. However, repeated testing and reselection resulted in a progressive increase in the number of symptomless plants in accessions 2N138-1, 2N23-3, 2N295-2 and 2N85. Within 2 years, several field and greenhouse selections from 2N138 and 2N23 were found to be uniformly immune and symptomless, respectively, in the greenhouse. Accession 2N138 was also immune to the 'necrotic' strain in the field and the greenhouse tests. Additional selections were heterogeneous with several plants resistant to both strains.

An immune and a highly resistant faba bean inbred line were crossed reciprocally with each of the two susceptible inbred lines. Results from testing $F_1$, $F_2$ and $F_3$ generations at $27^{\circ}$ C indicated that the resistance of line 2N23-2GH-3-2 to the 'mosaic' strain and the immunity of line 2N138-1GH-3-5 to both virus strains were each controlled by two recessive genes. One of the two genes for susceptibility in line UMFB13-1GH-1-3 was masked in the greenhouse and expressed only in the field. Symptoms in many $F_2$ plants appeared 14 days after inoculation,

but the fact that symptoms did not appear until 21 or 28 days after inoculation in some other $F_2$ plants was attributed to the heterozygous condition of a dominant gene.

Temperature had little effect on the time required for virus or symptoms to appear in the host. Higher inoculum concentration or four or eight pea aphids (Acyrthosiphon pisum Harris) were required to infect plants of line 2N23-2GH-3-2 with the 'mosaic' strain. The immunity in line 2N138-1GH-3-5 was overcome in the greenhouse when plants were inoculated by exposing them to viruliferous aphids or when epidermal strips were removed from leaves before mechanical inoculation. However, inbred lines and field selections from 2N138 were immune when viruliferous aphids were used to inoculate the plots in the field.

# GENERAL INTRODUCTION

Faba bean (Vicia faba L.) is an important legume crop in Western Canada and in many other parts of the world. It is primarily used as a protein source in the human diet and animal feed and occasionally as a green manure crop.

Several plant viruses, including bean yellow mosaic virus (BYMV), have been reported to infect field-grown faba beans and limit their production (Bos, 1970a; 1981). BYMV occurs commonly in the province of Manitoba and has been recognized as a potential threat to faba bean production (Bernier, 1975). Significant reductions in yield resulting from BYMV infection in faba bean have been reported (Heathcote and Gibbs, 1962; Izadpanah et al., 1969; Kaiser, 1973). Frowd and Bernier (1977) found that severe early BYMV infections could result in yield losses as high as 96.3%.

In the field, BYMV is readily transmitted by aphids in a non-persistent manner, has a wide range of hosts among legumes and non-legumes which include several perennial wild legumes such as clovers and vetches that are reservoirs of the virus (Bos, 1970a; Edwardson, 1974).

The control of the disease lies in eradicating the primary sources of inoculum for the virus, in controlling insect vectors and producing cultivars with resistance to the virus. The first two measures may continue to play a significant role in an integrated disease control program. However, both measures involve a continuous and timely use of

chemicals and must be repeated year after year. Breeding for resistant plants provides the most efficient and effective method of virus control (Holmes, 1954). Diseases of faba beans have received very little attention in the past and limited attempts to find host resistance to BYMV have not been successful (Nitzany and Cohen, 1964; Kaiser et al., 1968; Fiederow, 1980). However, resistance to BYMV has been found and successfully used in peas (Yen and Fry, 1956; Johnson and Hagedorn, 1958; Schroeder and Provvidenti, 1962, 1971; Barton et al., 1964), in Phaseolus beans (Baggett, 1956; Baggett and Frazier, 1957; Provvidenti and Schroeder, 1973), in cowpeas (Reeder et al., 1972) and in soybeans (Provvidenti, 1975). In preliminary studies, some 360 faba bean accessions from different regions of the world were screened at the University of Manitoba and resistance to BYMV was identified in several segregating accessions (C. Bernier, unpublished data). In the present studies, the accessions previously identified as having some resistance were subjected to more rigorous evaluation and selection under different environmental conditions. The genetic basis of virus resistance was then examined in selected faba bean inbred lines.

The expression of symptoms in BYMV-infected plants is influenced by temperature and possibly by the quantity of virus introduced into plants. In the field, faba beans are repeatedly exposed to the virus depending on numbers and movement of the aphids. Therefore, the effects of two temperature regimes and varying inoculum densities on the reactions of selected inbred lines were also studied.

# LITERATURE REVIEW

## 2.1 The Host

### 2.1.1 Faba Bean: A Brief History

Faba beans (*Vicia faba* L.) now grown in many regions of the world (Bond, 1976) are believed to have originated in the Near East (Cubero, 1974; Zohary and Hopf, 1973). The ancestry of *V. faba* is not known. *Vicia narbonensis* L. which was once considered to be the ancestor was found to be widely divergent cytogenetically (Zohary and Hopf, 1973). Similarly, *Vicia galilea* Plitm. et Zoh. has also been proposed to be the ancestor of *V. faba* because of its resemblance but Ladizinsky (1975) found that the albumin profiles of seed protein from *V. galilea*, *V. narbonensis* and *V. hyaeniscyamus* Mout. had little in common with the profile of *V. faba*. This, along with the fact that these species are cross incompatible with *V. faba* suggests that they are not the ancestors of faba beans (Conner, 1981).

Faba beans are thought to have been cultivated first in late Neolithic era (Zohary and Hopf, 1973). Their use has been largely confined to temperate zones and to the cool season in subtropical areas. The crop is grown extensively throughout the Middle East and China and also, to a lesser extent, in Southern Europe (Hawtin and Stewart, 1979) and Western North America (Evans and Slinkard, 1975). *Vicia faba* has been divided into three subspecies based on the seed size: *major*, *equina* and *minor* (Robinson, 1968; Evans and Slinkard, 1975). These subspecies include large, medium and small seed types, respectively. All these

subspecies are used for animal and human consumption (Cepeda, 1981). Small-seeded faba beans, however, have been found to be an excellent silage source and their ability to fix nitrogen in combination with suitable rhizobia makes them a good green manure crop. The small seed types are also preferred in Canada because seed can be manipulated with conventional cereal equipment and seed coats are lower than for large-seeded types (Crafts, 1979). Field trials have shown that the crop is well adapted to the moister growing areas of the Prairies (Evans et al., 1972). Current research is aimed at improving traits such as shattering resistance, earlier maturity and disease resistance (Furgal and Evans, 1976). Among other pathogens, bean yellow mosaic virus has been found to be quite prevalent and is regarded as a potentially serious pathogen in the province of Manitoba (Bernier, 1975; Frowd and Bernier, 1977).

### 2.1.2 Faba Bean Uniformity

Studies by Toynbee-Clark (1974) and Poulsen (1975) have indicated that V. faba is subject to cross-fertilization ranging from 19-79%. This large disparity found in outcrossing in different lines has been attributed to a number of factors such as the bee activity, flower colour and flowering time (Poulsen, 1975; De Vries, 1978). Plant density may also have an effect on the degree of cross-fertilization, although the evidence is conflicting (Holden and Bond, 1960). Poulsen (1975) found that earlier formed flowers tend to outcross more than later ones. However, the late flowers ensure higher seed production even under low bee activity. Some type of self-incompatibility controlled by a number of deleterious recessive genes that favour cross-fertilization has been proposed by Rowlands (1960). There is little evidence to support this theory, although

Holden and Bond (1960) have shown that after several generations of inbreeding the fertility declines even when there is selection for self-fertility. Poulsen (1975), on the contrary, suggests that fertility declines for the first few generations of selfing and later levels off as genes controlling autofertility become fixed. Nevertheless, the evidence that flowers do require tripping to produce pods (Rowlands, 1960; Toynbee-Clark, 1974) supports the fact that cross-pollination is favoured. To limit the amount of cross-pollination for inbreeding selections, an understanding of isolation distances is required. Pope and Bond (1975) found that an isolation distance of 134 m reduced cross-fertilization to 1-2%, while low levels of contamination could still occur at distances of 1.1 km or more.

### 2.1.3 Faba Bean as a Host to BYMV and Other Viruses

Presently, 16 different viruses have been reported from field-grown faba beans in various parts of the world (Bos, 1981). In addition, *V. faba* L. has been used as a test plant in research and found to be susceptible to a number of other viruses. The faba bean crop is thus potentially vulnerable to virus diseases. In fact, in the early 1920's, with a rapidly increasing interest in mosaic diseases (a name at that time equivalent to virus diseases), mosaic-diseased faba bean crops were among the first to attract the attention of virologists as in the U.S.A. (Dickson, 1921; Elliot, 1921), the Netherlands (Vandermeulen, 1927), Germany (Boning, 1927), Bermuda (Ogilvie, 1928) and Japan (Fukushi, 1930). Soon thereafter, mosaic diseases of faba bean were reported in many other countries (Quantz, 1953). It was later observed, however, that viruses not only cause mosaic, but various other symptoms as well. Stubbs

(1947) in Australia, described a virus that caused a destructive vascular wilt of broad beans. The virus now known as broad bean wilt virus is reported to be widespread in various parts of the world as a very damaging pathogen of several crops, including non-legumes and even woody ornamentals (Taylor and Stubbs, 1972). Gradually, other viruses such as alfalfa mosaic virus and cucumber mosaic virus were shown to cause diseases of faba beans (Bos, 1981). Within bean yellow mosaic virus, special strains have been detected that cause severe necrosis in faba beans (Bos *et al.*, 1974; Frowd and Bernier, 1977).

Knowledge of mechanisms by which viruses are transmitted facilitated host range studies which in turn helped to differentiate viruses and strains of a virus. It also provided an insight into knowing ways of natural spread and ecology of diseases concerned (Bos, 1981). Thus, Quantz (1950, 1953) was the first to detect seed transmission of a special mosaic virus of broad bean which he called Echtes Ackerbohnenmosaik virus (broad bean mosaic virus). Up to 15% of the seeds from diseased plants were found to carry the virus. Six of the 16 viruses reported in *Vicia faba* are now known to be transmitted through seeds obtained from naturally infected faba beans. Bos (1981) has also described some of the characteristics of the pertinent viruses that have so far been isolated from naturally infected faba beans. The information summarized by Bos (1981) was obtained from the studies of Inouve (1969) who reviewed reports on viruses detected on legumes including *Vicia faba* in Japan, from Cockbain (1980) and Sjodin (1980) who provided information on the incidence and importance of viruses infecting faba beans in England and Sweden, respectively, from Fiedorow (1980) in Poland and Schmidt *et al.* (1977a) in East Germany.

In Canada, Bernier (1975) first noticed that viral infections could potentially lead to severe yield losses in faba beans. Frowd and Bernier (1977) later isolated a virus characterized as BYMV and reported two strains from the field-grown faba beans of Manitoba. They also observed that pea aphid (Acyrthosiphon pisum Harris) was the chief vector for the virus spread in the fields. In other parts of the world, other aphid species are also known to transmit the virus.

Multiple infections with other viruses and completely different pathogens are now known to complicate symptoms and detection of pathogenic viruses in V. faba. In complex infections, interactions between viruses may range from antagonism to synergism. Viruses that are normally latent in plants may greatly aggravate the effect of other viruses (Bos, 1981). Symptoms of virus diseases may easily be mistaken for symptoms of diseases caused by other pathogens. Certain fungi may only attack plants already virus-infected or may be more pathogenic in virus-infected plants. Leaf roll-diseased faba bean plants are more susceptible to Botrytis fabae (Tinsley, 1959) and root rot inducing fungi (Kaiser, 1973).

## 2.2 The Virus

Bean yellow mosaic was first recognized by Pierce (1934) in a bean plant of Red Vallentine variety grown in the fields of the University of Wisconsin, Madison. The causal agent was called yellow bean mosaic virus or bean virus 2 by Pierce, but was later more often referred as bean yellow mosaic virus (BYMV) or more technically BYMV */* : */* : E/E : S/Ap. The virus is now known to occur in many countries and is evidently worldwide in distribution (Bos, 1970a, 1981). The names, pea mosaic virus (Doolittle and Jones, 1925), bean virus 2 (Pierce, 1934), Phaseolus virus 2 (Smith, 1937) and gladiolus mosaic virus (Smith and Brierley,

1944) have been used as some of the synonyms for the virus (Bos, 1970a).

The virus has flexuous particles about 750 nm long, is readily transmissible by sap inoculation and by aphids in a non-persistent manner and causes diseases in economically important plant species such as peas, french beans, faba beans, clovers, gladiolus and in several other species. It can also infect a number of wild legumes and non-legumes such as Liliiflorae (Bos, 1970a). The literature on host range is further reviewed in subsection 2.2.3.

The stability of the virus depends greatly on virus source, test plant and conditions. The expressed sap loses most of its infectivity after heating 10 min at 60° C and depending on the virus source and strain, the thermal inactivation temperature is between 50 and 70° C. The dilution end point is usually between $10^{-3}$ and $10^{-4}$ and aging *in vitro* at room temperature is 1-7 days (Bos, 1970a).

### 2.2.1 Transmission

More than 20 aphid species have been reported to transmit the virus (Kennedy *et al.*, 1962). The important aphid species include *Acyrthosiphon pisum*, *Macrosiphum euphorbiae*, *Myzus persicae* and *Aphis fabae*. *A. pisum* biotypes have been found to differ greatly in transmission efficiency (Sohi and Swenson, 1964). The loss of transmissibility by some isolates has not been found to be related to repeated sap transmissions (Swenson *et al.*, 1964; Kamm, 1969). Transmission of virus by aphid species in the field may depend upon the geographical area and the virus strains involved. In Manitoba, the pea aphids which is the principal aphid species colonizing *Vicia faba* has been found to be a vector of BYMV (Frowd and Bernier, 1977).

Although seed infection is uncommon, the virus has been reported to

be occasionally seed-borne in faba bean by Zschau (1961), Evans (1973) and Kaiser (1973). Seedlings from such infected plants, therefore, have the potential to be primary foci of disease outbreaks. The findings of some other workers (Grogan and Walker, 1948; Zaumeyer and Fisher, 1953; Frowd and Bernier, 1977; Drijfhout, 1978), however, do not agree that the virus is seed-borne. Out of 20 broad bean lines from nine countries, Kaiser (1973) detected 0.1-0.9% BYMV infection in seeds of 12 lines, while Cockbain et al. (1976) did not see a single case of seed-borne infection in 30 crops of spring-sown faba beans grown between 1970 and 1976 in England. Only two infected in about 60 samples (0.2%) of seeds grown in a glasshouse were detected.

### 2.2.2 Symptoms

Symptoms caused by BYMV vary greatly with type and variety of plant and the virus strains involved. The earlier literature mentions that symptoms like yellow mosaic (Pierce, 1934), necrotic local lesions (Zaumeyer and Fisher, 1953), stem or top necrosis (McWhorter and Boyle, 1946) and pod-distortion (Grogan and Walker, 1948) may be produced in susceptible bean varieties. In Vicia faba the virus is reported to cause a mild mosaic with irregular dark green islands with a slight malformation of young leaves and stunting of plants. Characteristically, the virus produces crystalline inclusion bodies in infected plant cells (McWhorter, 1941). Later, Zaumeyer and Goth (1963) reported isolation of a red clover necrosis virus isolate that produced brown primary necrotic lesions followed by systemic leaf necrosis, stem streaking and death of plants. More recently, BYMV isolates were also obtained that produced local and systemic necrotic lesions followed by tip and/or vascular necrosis and rapid collapse of susceptible faba bean plants (Frowd and

Bernier, 1977).

On pea plants (Pisum sativum), Doolittle and Jones (1925) reported a disease that characteristically resulted in a bright yellow mosaic and considerable distortion of plants. The virus then named pea mosaic virus was later identified as a strain of bean yellow mosaic virus (Bos et al., 1974).

On white sweet clover (Melilotus alba), symptoms of BYMV first appear as small light yellow spots on the leaves. The spots then usually enlarge and coalesce with others producing small light green blotches interspaced with dark green areas followed by slight dwarfing and ruffling of leaves in severe cases of infection (Smith, 1972). Symptoms produced on other legume and non-legume plants are further discussed under host range studies.

### 2.2.3 Host Range

Since the first description of BYMV by Pierce in 1934 (Bos, 1970a), many isolates with widely varying host ranges have been reported (Kovachevsky, 1968; McCord and Gudauskas, 1968; Bos, 1970b; Bos et al., 1974; Edwardson, 1974; Jones and Diachun, 1977; Hampton et al., 1978). In a comprehensive study on host ranges, Kovachevsky (1968) found 77 leguminous and three non-leguminous species (opium poppy, Circium arvense and Chenopodium album) as natural hosts of BYMV in Bulgaria, 45 of them being new hosts. Of the tested legume species, only Lotus corniculatus and L. conjugatus were found immune to the virus, while certain Trifolium species and Melilotus species were found to serve as its winter hosts. Bos (1970a) gave a summary of host range and symptomatology of BYMV isolates that infect not only leguminous species but certain non-leguminous species as well. He divided hosts into three categories as follows:

Propagation species which serve as host to propagate virus and maintain the cultures are: Pisum sativum, Vicia faba, Phaseolus vulgaris and Nicotiana clevelendii.

Diagnostic species which provide an aid in diagnosis of virus by presence of inclusion bodies of typical morphology and localization are: Phaseolus vulgaris, Pisum sativum, Vicia faba, Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Nicotiana tabacum, Spinacia oleracea and Tetragonia expansa.

Assay species that have been used to measure the relative titres of virus are C. amaranticolor, C. quinoa and for some BYMV strains, Crotaloria spectabilis, Phaseolus aureus and some Kenland red clover clones.

Based on this information, Bos et al. (1974) selected a list of hosts that could be used to differentiate isolates of bean mosaic, pea yellow mosaic and pea necrosis strains of BYMV, pea necrosis virus and pea seed-borne mosaic virus. The differentials used were Phaseolus vulgaris vars. Double White Princess and Great Northern 123; Pisum sativum vars. Koroza and Juwel; Vicia faba var. Compacta, Chenopodium amaranticolor and C. quinoa. The choice of host species or varieties used as differentials, however, could vary from one person to another depending upon geographical area and the virus isolates being studied. Hence, the set of differentials used by Thomas and Zaumeyer (1953), El-Attar et al. (1971), Jones and Diachun (1977), Frencel and Pospieszny (1977) and Allam et al. (1979) differed not only from that of Bos et al. (1974) but also from each other. For lack of standard identification criteria, therefore, similar or identical strains could be reported by different workers and given different names. To solve this problem and to promote international

communication and cooperation among plant pathologists working with legume viruses, a group called 'The International Working Group on Legume Viruses' was established in 1962 (Hampton et al., 1978). The group comprised eight investigators located at different places in the world and carried out a comprehensive test to study the reaction of 23 selected plant hosts against 38 legume viruses in an effort to standardize host range and reaction for type cultures of important legume viruses of northern temperate zones on uniform plants. Seedlots of plant hosts were gathered at one location, subdivided into eight equal quantities for distribution to the eight investigators. Thus, each investigator tested documented isolates of viruses on plant host germplasm identical to that employed by other investigators. An earlier working group published a revised list of host plants proven useful for definitive work with legume viruses (Vanderveken, 1976). Based on 1700 symptomatological and host range data, the group organized by Hampton et al. developed a key that could be used to identify and distinguish 38 viruses. Although the information for structuring the key was quite comprehensive, some limitations were recognized (Hampton et al., 1978). A major limitation was that with the exception of five viruses: alfalfa mosaic virus, BYMV, red clover vein mosaic virus, white clover mosaic virus and pea stunt virus, only one isolate of the other 33 viruses was used while different strains are known to induce different symptoms on the same set of hosts. For this reason, the authors believe that final identification of virus and strains should be confirmed by serology and, where possible, by other properties of the virus particles. In the host range analyses, it was found that BYMV could infect as many as 11 of the 23 hosts tested. The symptoms included local chlorotic spots to necrotic spots, vein clearing and severe mosaic coupled in some hosts with severe stunting and necrosis of stem tissue.

2.2.4 Strains of BYMV

Since 1934, several strains of BYMV have been reported from different areas of the world. The work by Doolittle and Jones (1925), Pierce (1935) and Murphy and Pierce (1937) led to the belief that (common) pea mosaic virus (PMV) that also causes mosaic in broad beans (Boning, 1927; Merkel, 1929), mottling in red clover (Zaumeyer and Wade, 1935) and 'sore shin' in yellow lupin (Chamberlain, 1935) is a distinct entity from BYMV. One of the main distinctions between BYMV and PMV was that the later virus was not able to infect beans (Phaseolus vulgaris). However, it was occasionally reported that PMV could infect beans (Zaumeyer and Wade, 1935; Johnson and Jones, 1937), although it was much less pathogenic to bean than normal BYMV (Schroeder and Provvidenti, 1966; Taylor and Smith, 1968).

Serologically, Goodchild (1956b) and Taylor and Smith (1968) could not distinguish between BYMV and typical isolates of PMV. Genetic studies done by Barton et al.(1964) showed that resistance in pea, especially Perfection type varieties, to isolate of BYMV (BYMV-B25) and PMV (F210) was conditioned by the same genotype. These findings, therefore, support the argument that PMV is only a strain of BYMV.

Grogan and Walker (1948) reported a strain that could infect Idaho Refugee bean and other varieties resistant to the type strain (Pierce, 1934). The pods on infected plants are severely warted, misshapen and disfigured. This strain differed from the type strain and other strains in the sense that it does not infect broad beans, scybeans, tobacco and sweet clovers (Thomas and Zaumeyer, 1953).

In Oregon (USA), McWhorter and Boyle (1946) discovered a black root x-disease strain of BYMV which caused a variable symptom in Phaseolus

vulgaris. The most frequent symptoms were purpling of leaf bases of lower leaves accompanied by drying of tissues within an often premature death of plants. At maturity, some pods had black inner walls, while in other cases, plants developed root rot that extended up into the stem. Plants so affected wilted and died prematurely. The strain has not been reported from other parts of the world.

A necrotic lesion strain which produced solid brownish red necrotic local lesions on beans was reported by Zaumeyer and Fisher (1953) in Eastern Washington. Besides the local lesions, the virus produced more intense mottling symptoms on some bean varieties than those produced by the type strain of the BYMV. Top necrosis which was produced by the pod-distorting and x-disease strains seldom develops with this strain. Phaseolus lunatus, Vigna sesquipedalis and V. sinensis were susceptible to the necrotic lesion strain, but were resistant to other strains. In addition, this strain infected Nicotiana tabacum and N. rustica which differentiates it sharply from the type virus, the pod-distorting and the x-disease strains. El-Attar et al. (1971) and Allam et al. (1979) obtained some isolates from infected broad beans in Egypt that resembled this strain very closely in terms of host reaction, physical properties and serology.

Rex and Zaumeyer isolated a strain, termed as severe yellow mosaic strain from Idaho in 1953. The symptoms caused by this strain were top necrosis and death of certain bean varieties and a severe yellow mosaic in others. No infection was obtained outside the leguminosae except for yellow necrotic lesions on N. tabacum, N. rustica and N. sylvestris.

Another virus so far considered to be a strain of BYMV based on host range, symptomatology and some physical characters is sweet pea streak

virus described by Ainsworth (1940). The strain caused a well defined vein clearing followed by a distinct but not very severe mottling of young sweet pea leaves and necrotic streaks on the stem and petioles; reddish brown local lesions followed by vein-clearing of youngest leaves in Vicia faba. On P. vulgaris the virus caused a few local lesions consisting of pale green spots each surrounded by a fine necrotic rim. Later a more general veinal chlorosis developed, growth ceased and the plant died within 4-6 weeks.

A new strain of BYMV that produced a stem streaking on peas similar to that caused by the type pea streak virus was isolated by Zaumeyer and Goth (1963) from the infected red clover plants showing large spreading necrotic spots and vein necrosis. The strain called red clover necrosis virus, killed plants of stringless Green Refugee and several other varieties of beans about 2 weeks after inoculation. All pea varieties tested, except Perfection type, were susceptible. In general, host range and physical properties were similar to that reported for other BYMV strains (Zaumeyer and Goth, 1963).

Bos (1970b) described the presence of three new viruses in the Netherlands. They were westeria vein mosaic virus, pea necrosis virus and pea leaf roll mosaic virus. These viruses resembled BYMV type strain B25 in particle size and morphology and in host reaction in certain plants, but differed in host range and reaction in other plants. These viruses were considered different despite the fact that they were all related serologically to BYMV. Later, Bos et al. (1974), pointing to the problem of classifying BYMV isolates, divided them into three distinct groups based on host ranges, symptoms and bean and pea varietal reactions; serologically the groups did not differ appreciably. Thus, isolates

E212, L1 and B25 represented typical BYMV; isolates E198, E204, KOW28 represented pea yellow mosaic virus strain and isolates E197, E199 and E221 represented pea necrosis strain of BYMV. Pea necrosis virus (E178) of Bos (1970b) remained a distinct entity, while pea mosaic virus was considered only a strain of BYMV grouped now under pea yellow mosaic strain. Thus, each group had isolates differing slightly from one another in host range or reaction, but could still be called a same strain. The difference, however, between pea necrosis strain and pea necrosis virus was not clear. There is practically no difference in host range, while in host reaction, pea necrosis strain is closer to pea necrosis virus than to BYMV. Pea necrosis strain and PNV both cause tip or systemic necrosis of peas or beans, while BYMV causes mainly mosaic type symptoms. The authors (Bos et al., 1974) found that cross-protection tests, serological relationships and knowledge of physical properties were of little help in differentiating strains, since according to them, these characteristics relating to protein coat need not necessarily parallel pathogenic differentiation. Beczner et al. (1976) further studied relationships between PNV and BYMV isolates and discovered that some isolates were intermediate between PNV and BYMV, serologically and biologically, as also were other legume viruses such as bean common mosaic virus, pea-seed-borne mosaic virus, clover yellow vein virus (Beczner et al., 1976) and some other members of the potyvirus group (Bos et al., 1977).

Two strains 'M' and 'S' resembling some isolates of BYMV of Bos et al. (1974) were found to infect faba beans in Manitoba fields (Frowd and Bernier, 1977). The strain 'M' resembled closely group I isolates, while strain 'S' identified to be a pea necrosis variant of BYMV resembled isolates of group IIb of Bos et al. (1974). Unlike group IIb

isolates, this strain, however, has similarity with the BYMV strain isolated from P. vulgaris which produces visible necrotic and systemic lesions in V. faba (Zaumeyer and Fisher, 1953).

Thottapilly et al. (1976) isolated a virus from infected broad beans in Michigan that was described to be a strain of BYMV based on its serological relationship. The virus causes mosaic and a characteristic upward rolling of young infected pea and broad bean leaves, usually in one plane; in P. vulgaris it causes mosaic, stunting and leaf-malformation such as curling. It does not cause systemic symptoms in Chenopodium amaranticolor or C. quinoa; only local lesions are produced in both species. Its relationship with pea leaf-rolling virus from Czechoslovakia (Musil, 1966; Kvicala and Musil, 1967), pea leaf roll mosaic virus from the Netherlands (Bos, 1970b) or pea leaf roll virus from Iran (Kaiser and Danesh, 1971) have not been studied.

Bos et al. (1974) found that BYMV isolates belonging to the three different groups did not differ appreciably in serological properties. However, work done by others (Granett, 1974; Jones and Gardon, 1974) suggested that serological distinctions exist between the members of BYMV group. In a study to determine the biological and serological relationships between the BYMV isolates obtained from different sources and among isolates obtained from infected white and red clover plants in the USA, Jones and Diachun (1977) demonstrated that all virus isolates could be classified into three distinct groups based on reaction by differential hosts and serology. Thus, isolates that caused yellow mosaic, necrosis and death in peas and faba beans, tip necrosis and death in beans and infection in Burley 21 tobacco were put under group I and the group comprised mainly of clover yellow vein virus (ClYVV) and

BYMV isolates from white clover. Group II isolates which correspond to B25 of Bos et al. (1974) caused mild light-dark green mosaic in pea and faba beans and moderate mosaic in beans. Group III isolates generally did not infect tobacco, but infected susceptible peas and faba beans causing either mild light green mosaic or stronger yellow-green mosaic but no necrosis or death; they also caused mild mosaic, chlorotic spotting or no symptoms in bean cultivars. Pea mosaic virus isolates belonged to this group. The authors were also able to observe serological distinctions in these subgroups. Group I was serologically distinct and did not cross-react in Ouchterlony double diffusion tests, Group III generally, and Group II, occasionally, developed spurred cross-reactions with Group I. Hence, they claimed that serology as correlated with the key host reactions was still a reliable tool for classifying BYMV isolates.

The difficulties involved in classifying legume viruses in general and BYMV in particular probably reflect the relatedness among legume plants or the evolution of different legume species vis a vis their viruses. The literature, however, is replete with information that BYMV is a variable pathogen and knowledge of pathogenic variations among its isolates and the range of symptoms produced by them is very desirable for a successful breeding program for resistance.

## 2.3 Genetics of Resistance

In view of the economic importance of faba beans as a commercial crop in many parts of the world, attempts have been made to find sources of resistance in faba beans to BYMV infection (Nitzani and Cohen, 1964; Kaiser et al., 1968; Fiedorow, 1980), but without success. However, resistance to BYMV has been established in many other legumes and has been the subject of genetic studies since the mid 50's.

The genetics of resistance to BYMV was initially studied in Germany by Rudorf (1955). He made an interspecific cross between Phaseolus vulgaris and P. coccineus which were highly susceptible and immune to BYMV, respectively. The studies, however, remained incomplete since the $F_1$ generation was largely sterile. Using the same parents, Baggett (1956), on the other hand, was able to obtain fertile $F_1$ progenies and thus tested a large number of $F_2$ plants against Y and W strains of BYMV. Strain Y resembled the pod-distorting strain of Grogan and Walker (1948), while strain W, obtained locally, was more severe. The data obtained from $F_1$ and $F_2$ populations did not produce clear segregation patterns. However, they suggested that resistance was recessive and apparently conditioned by at least two and possibly three 'major' genes. Effects of some modifying genes were also noticed in terms of a continuous variation in expression of visible symptoms.

In New Zealand, Yen and Fry (1956) tested $F_1$, $F_2$, $F_3$ and backcross populations from crosses involving two mosaic susceptible and two immune pea varieties against a pea mosaic virus strain of BYMV and proposed that the immune reaction found in William Massey and Onwald varieties was controlled by a single recessive gene. The gene was designated as 'mo'. By timing the appearance of mosaic symptoms and studying symptomatology, the authors also stated that susceptible heterozygotes could be separated from the homozygotes. Plants that showed mosaic symptoms in 7 days after inoculation were largely homozygous, while most of those that showed mosaic later were heterozygous.

Baggett and Frazier (1957) studied inheritance of resistance to two strains of BYMV obtained from Oregon State. Northern U.I. 31, a resistant bean variety, was crossed with two other susceptible bean

varieties, O.S.C. 21 Blue Lake and Montana Great Northern 43-15. The data from segregating $F_2$ progenies showed a pattern similar to that reported by Baggett (1956). Resistance was recessive and was governed by two or possibly three 'major' genes with modifiers influencing type and severity of symptoms.

Johnson and Hagedorn (1958) tested progenies of crosses between two susceptible and four resistant pea varieties and concluded that in all four resistant varieties, resistance was conditioned by a single recessive gene. The deviations from predicted ratios in $F_2$, $F_3$ or $F_4$ progenies were attributed to delayed symptom appearance in some susceptible heterozygotes. However, the reactions were studied at 15-20° C only.

Schroeder and Provvidenti (1962) also identified the gene 'mo', which conditioned resistance in the pea variety Bonneville and reported a new mild strain of BYMV that could overcome resistance found in this variety to other virus isolates.

Barton et al. (1964) carried out genetic studies on inheritance of resistance and susceptibility in garden peas to two BYMV strains, BY2 that caused dark-light mottle and PV2 that caused bright yellow mottle in peas. By testing $F_1$, $F_2$ populations and clones of $F_2$ families, they established that the gene 'mo' found in pea line G11 governed resistance to both virus strains and that high temperature influenced the expression of host reaction to both strains. A similar study done by Schroeder and Provvidenti (1971) revealed that the gene 'mo' in the pea variety Bonneville conferred resistance not only to BYMV but to watermelon mosaic virus-2 as well and that the relationship of temperature and expression of symptoms was similar in both virus-host systems. Higher temperature (28° C) produced earlier and more severe symptoms than the lower temperature (16° C).

Diachun and Henson (1965, 1974) studied inheritance of a necrotic reaction induced by an isolate of BYMV in red clover plants. Clones of Kenland red clover were selected for their distinct differential reactions, i.e. mottling, necrotic spotting and complete resistance and crosses were made between clones having different combinations of reactions. Because of self incompatibility found among $F_1$ plants, $F_2$ plants were not available and only $F_1$ plants were studied. The results were explained as follows: A single dominant gene 'N' conditions necrotic spotting in red clover. Another dominant gene 'R' that confers resistance (symptomless) is dominant to mottling and epistatic to necrotic spotting. Thus, the $F_1$ clones that showed mottling reaction had nnrr genotype, those that segregated into 1:1 for resistant and mottling reactions had nnRR and those that segregated into resistant, necrotic spotting and mottling reactions had NnRr genotypes. This was thus the first evidence that a necrotic reaction to a BYMV isolate was controlled by a single dominant gene. Resistance to BYMV conditioned by a dominant gene was also reported by Schroeder and Provvidenti (1968). Progenies of $F_1$, $F_2$ and reciprocal backcrosses between bean varieties were studied against a common pea mosaic strain of BYMV (same as PV2 of Barton *et al.*, 1964) and it was found that resistance in Red Kidney was governed by a dominant gene designated as By.

Further evidence that resistance to BYMV might be controlled by a dominant gene came from the findings of Dickson and Natti (1968). They developed a resistant selection originating from a cross of *P. vulgaris* var. Blue Lake x *P. coccineus* and crossed it with five susceptible bean lines. They identified a single gene for resistance to a mosaic producing strain of BYMV in this selection and named it By-2.

A third gene associated with resistance to BYMV which was recessive was later recognized in a bean variety, G.N. 1140, by Provvidenti and Schroeder (1973). $F_1$, $F_2$, $F_3$ and backcross populations from crosses between G.N. 1140 and Bernal or Black Turtle bean varieties provided data to reveal that resistance in G.N. 1140 was inherited through a single recessive gene designated By-3. The authors observed that discrepancies in results from those of Baggett and Frazier (1957) who reported more than one gene involved in determining resistance in bean variety G.N. 1140 was probably due to different conditions or different virus pathotypes.

Reeder et al. (1972), working with cowpeas, reported a high level of resistance to a cowpea strain of BYMV in P.I. 297562, a cowpea (southern pea) introduction to United States. Its mode of inheritance was studied by crossing it with three susceptible cultivars: Knuckle Purple Hull, Mississippi Silver and Princess Anne and an Alabama breeding line Ala 5623-1-2. Results of $F_1$, $F_2$ and backcross tests showed that resistance in P.I. 297562 was governed by a single recessive gene.

## 2.4 Differentiation of Genotypes by Temperature Manipulation

Yen and Fry (1956) had shown that pea plants heterozygous with 'Momo' genotype delayed symptoms due to BYMV. The observations were later confirmed by Johnson and Hagedorn (1958). However, that symptoms in heterozygous plants inoculated with certain strains of BYMV can be manipulated by changing the temperature was first demonstrated by Schroeder et al. (1960). They observed that heterozygous pea plants with 'Momo' genotype developed symptoms as quickly as homozygous susceptible plants with 'Momo' genotype when incubated at 26.6° C or higher temperatures and symptoms disappeared when infected plants were returned to 18° C or lower temperature. The converse was also found to be true. Later isolates of BYMV

were obtained which could also overcome resistance conditioned by the 'momo' genotype at 26.6° C or higher (Provvidenti and Schroeder, 1963). Schroeder and Provvidenti (1966) later observed that at 18° C or lower, some pea mosaic strains of BYMV did not infect 'Momo' genotypes obtained from crosses involving G385, Perfection and New Era pea varieties, while those strains infected and caused symptoms at 27° C or higher.

The effect of temperature on host reaction to viruses has also been observed in other host-virus systems, e.g. in tobacco due to infection by tobacco ring spot virus (Valleau, 1941; Benda and Naylor, 1958; Hendrix, 1972), in potato due to infection by potato virus X (Venekamp and Beemster, 1980) and in tomato due to infection by TMV (Schroeder et al., 1967; Cirulli and Alexander, 1969). However, little is known about the effect of temperature on the expression of symptoms in heterozygotes. Two contrasting observations are available in tobacco-TMV interactions. According to Pelham (1972), when tomato plants heterozygous for Tm-2 gene for resistance to TMV were inoculated and grown at high temperatures (30-31° C), rather than producing only local necrotic spots, they segregated into plants that developed local lesions and plants that developed systemic necrosis. The phenotypic expression of heterozygous (Tm-2/+) plants was markedly influenced by temperature.

Pilowsky et al. (1981), however, found no evidence that the two types of reactions were the result of genetic differences in the parental material. The authors demonstrated that two types of reactions were also observed when differential concentrations of TMV were used to inoculate the heterozygous tomato plants at 30-31° C. Moreover, they also observed similar reactions, local and systemic necrosis, in some of the resistant homozygous tomato plants with Tm-2/Tm-2 genotypes inoculated with TMV. They suggested that certain minor or modifier genes might take part in

determining final host reaction.

Differentiation of heterozygous plants by temperature manipulation or by use of appropriate virus inoculum thus has practical application in breeding for resistance to viruses, especially the genotypes that are sensitive to changes in temperature and inoculum concentration.

## 2.5 Nature of Resistance to Viruses

Resistance in plants may be attributed to one of several factors such as i) plants may escape infection, ii) plants may be resistant to virus vectors, iii) plants may be genetically immune to virus infection, iv) they may allow only restricted multiplication or movement of virus in infected plants or v) resistance may be induced or acquired by the plants. Since no resistance has been found in faba bean to BYMV (Bos, 1981) and very little is known about the resistance mechanism in other legumes to this virus, the literature in other plant-virus systems was reviewed to provide an understanding of the mechanisms of resistance.

Thomas and Martin (1970) observed that resistance found in tomato breeding lines, VR Moscow and VF 145 to infection by curly top virus lies in their ability to escape infection and that resistance was expressed both in the field and greenhouse and was effective in both seedling and adult stages.

Khalf-Allah *et al*. (1973), working with cucumber mosaic virus, discovered that resistant/tolerant cowpea plants had only two-thirds of the amount of nitrogen and protein present in susceptible plants 15 days after inoculation, indicating that virus multiplication was much higher in susceptible plants than in resistant ones.

Kassanis *et al*. (1974) studied resistance to TMV infection induced in tobacco plants by preinoculation with one of the five viruses: PVY,

PVX, cucumber mosaic virus, potato acuba mosaic virus and alfalfa mosaic virus. They found that three proteins were present either in resistance-induced plants or in those healthy plants that had been injected with polyacrylic acid. These three unidentified proteins were absent in uninoculated or untreated tobacco plants. Both resistance and protein levels were decreased when the resistance-induced plants were kept at 32° C for 2 days before inoculation with TMV. Plants kept at 22-24° C before inoculation with TMV, showed no change in resistance or protein levels.

Maksoud et al. (1975) studied the nature of resistance to TMV in tomato plants i) by comparing virus concentration in resistant and susceptible plants 15 days after inoculation and ii) by attempting to detect an antiviral principal (AVP) in inoculated plants. They found that virus titres were nil in resistant plants and very high in susceptible plants suggesting that resistant plants suppressed virus multiplication rather than conferring tolerance to viral infection. They also discovered that resistant plants contained an AVP with more inhibitory activity to TMV than that extracted from susceptible plants in which the production rate of AVP was insufficient to suppress virus multiplication.

An accession of okra (Abelmoschua manihot) grown in India because it is highly resistant to yellow vein mosaic virus (Sandhu et al., 1974) was used to study the inheritance and the nature of resistance to this virus by Singh and Thakur (1979). They found that a resistant selection of A. manihot took 14 days longer to develop symptoms than a susceptible okra cv. Pusa Sawani or the $F_1$ plants obtained from the cross involving those parents. The virus concentration in resistant plants was 40% lower than in susceptible or in $F_1$ plants. The resistance, therefore, was expressed

via diminished multiplication of virus in A. manihot which, however, was a symptomless carrier of the virus.

Su et al. (1979) detected high levels of proteases, papain, chymotrypsin and carboxypeptidase in red clover clone, KyC 13, which was hypersensitive to BYMV infection compared to the clone, KyC 71-8, or KyC 36, that reacted with systemic necrosis or chlorosis, respectively, at 22° C. The activity of papain in susceptible clones reached the same level as in resistant clones at 32° C. The increased papain in susceptible clones was correlated with localization of virus in red clover clones at about 32° C.

# RESULTS OF RESEARCH

## 3.1 Resistance in Faba Bean (*Vicia faba*) to Bean Yellow Mosaic Virus


### ABSTRACT

Sixty-eight open-pollinated faba bean selections resistant to bean yellow mosaic virus (BYMV) in preliminary field experiments were evaluated further in the field from 1978 to 1981. The selections were inoculated with the 'mosaic' strain of BYMV, mechanically and by exposing to viruliferous pea aphids (*Acyrthosiphon pisum* Harris) in separate experiments. Most of the selections were heterogeneous for disease reaction. However, repeated testing and reselection resulted in a progressive increase in the number of symptomless plants in accessions 2N138-1, 2N23-3, 2N295-2 and 2N85-2. There was also a considerable increase in the number of resistant (symptomless and mild mosaic) plants in 1980 and 1981 in accessions 2N101-1, L5-22-1 and 2N425-3. One hundred and twenty-five selections from the 1978 field trials were further evaluated in the greenhouse in 1978 and 1979 and of these, six selections (2N138-1GH-3, 2N295-2GH-1, 2N23-2GH-3, L5-22-1GH-1, UMFB15-1GH-1 and L5-42-1GH-1) were symptomless. When superior selections were inoculated with the 'necrotic' strain, accession 2N138 was immune in both the field and the greenhouse. In addition, many accessions were heterogeneous with several plants having resistance to both virus strains.

# INTRODUCTION

Bean yellow mosaic virus (BYMV) is a common and destructive pathogen of faba beans (Vicia faba L.) in many areas of the world (El-Attar et al., 1971; Kaiser, 1973). The disease has been frequently observed in the province of Manitoba since faba beans were introduced in 1970 (Bernier, 1975). Yield losses as high as 96.3% have occurred as a result of severe early infections in faba beans (Frowd and Bernier, 1977). In Manitoba, transmission of BYMV in seed has not been demonstrated and the virus is principally transmitted from perennial forage legumes (clovers and vetches) to faba beans by pea aphids (Acyrthosiphon pisum Harris) (Frowd and Bernier, 1977).

Resistance, when available, is the most efficient means of control, however, earlier attempts by Nitzany and Cohen (1964), Kaiser et al. (1968) and Fiedorow (1980) to find resistance to BYMV in faba beans were not successful. Resistance to BYMV has been found and successfully used in peas (Yen and Fry, 1956; Johnson and Hagedorn, 1958; Schroeder and Provvidenti, 1962, 1971; Barton et al., 1964), in Phaseolus beans (Baggett, 1956; Baggett and Frazier, 1957; Provvidenti and Schroeder, 1973), in cowpeas (Reeder et al., 1972) and in soybeans (Provvidenti, 1975).

In view of the desirability of developing faba bean cultivars with resistance to BYMV, an evaluation of the faba bean germplasm collection at the University of Manitoba was made in the field from 1975 through

1977. Some 360 accessions from different regions of the world were evaluated in the field exposed to natural infestations of BYMV-viruliferous pea aphids (C.C. Bernier, unpublished data). Although none of the accessions appeared to be uniformly resistant to the virus, 68 accessions had several plants free from symptoms or with mild mosaic only. Faba beans are partially cross-pollinated and cultivars and lines are restricted populations of several genotypes (Toynbee-Clark, 1974; Poulsen, 1975). Since the incidence of BYMV throughout the experimental plots was high and the spread excellent among the susceptible cultivars spaced throughout the trial in each year, the variability in disease reactions within accessions appeared largely due to genetic resistance. However, some of the variability might also be due to preferential feeding by aphids or to uneven dispersal of aphids throughout the plots.

In this study, the accessions previously identified as having some resistant plants (symptomless or mild mosaic) were submitted to more rigorous evaluation and selection under conditions of mechanical and aphid inoculations in the field, followed by further testing and selfing in the greenhouse. Superior selections from the greenhouse were subsequently evaluated in the field to determine if greenhouse results correlated with the field results.

# MATERIALS AND METHODS

## Field Evaluation

During 1978, 68 accessions and selections resistant in preliminary experiments were evaluated in separate trials in which they were mechanically inoculated and exposed to viruliferous pea aphids. Due to limited seed quantities, single-row non-replicated plots were used except for 20 accessions which were replicated three times in a randomized complete block design in the mechanical inoculation test. Fifty-two resistant single-plant and bulk selections were made and evaluated again in 1979 when they were mechanically inoculated only, 22 more promising selections of these were replicated twice. Selections from the 1979 field trials were retested in 1980 and 1981 along with some of the superior inbred lines developed in the greenhouse during 1978 and 1979. Planting in all years was done by the end of May or early June at the University of Manitoba. The plots were located away from areas where natural infection was known to occur and surrounded by plots of cereal or rapeseed. Fifteen to 20 seeds per selection were planted in single-row plots, 1.5-m long and 60-cm apart. In tests where aphids were used to inoculate the selections, two rows of virus susceptible cultivar 'Diana' were planted at the ends of each plot to act as virus spreader rows.

## Greenhouse Evaluation

One hundred twenty-five selections made from the 1978 field trials for symptomless plants or plants showing only mild symptoms, were tested and reselected at 27$^{o}$ C in 1978 and 1979. Five to 10 scarified seeds per

selection were planted in clay pots 12-cm in diameter using a 2:1:1 mixture of soil, sand and peat moss. Fertilizer and/or insecticides were applied as necessary to ensure good growth and seed yield and freedom from aphids and mites. The selected plants were moved to controlled environment rooms with 21-15$^{o}$ C diurnal temperature and 16 hours photoperiod and allowed to self.

Virus Strains, Inoculum Preparation and Method of Inoculation

A mild strain 'M' and a severe strain 'S' of BYMV used by Frowd and Bernier (1977) and hereafter referred to as 'mosaic' and 'necrotic' strain, respectively, were used in testing field and greenhouse materials. Symptoms caused by the 'mosaic' strain ranged from vein clearing, leaf mottling, mosaic and bronzing to severe stunting, stem necrosis and premature pod splitting in more susceptible plants. The 'necrotic' strain caused severe mosaic, numerous minute dull brown necrotic spots in young leaves leading to tip or vascular necrosis and rapid death of more susceptible plants. The 'mosaic' strain is more common in the province of Manitoba and was used in all tests while the 'necrotic' strain was used to evaluate only the superior selections tested in the 1979 greenhouse and 1980 field trials.

For inoculum preparation, young BYMV-infected leaves of greenhouse-grown plants of the cultivar 'Diana' were harvested 6-8 or 10-14 days after inoculation with 'necrotic' or 'mosaic' strain, respectively. Leaves were ground in 0.1 M phosphate buffer pH 7.4 (1:10, w/v), the sap was filtered through two to four layers of cheesecloth and was mixed with carborundum (600 mesh) at 2% (w/v).

In the 1978 field trials, accessions were mechanically inoculated

by rubbing the freshly prepared inoculum on four young leaves of 1-month-old test plants using a cotton swab or pipe cleaner. The leaves were washed with water immediately after inoculation. In 1978 and 1981, selections were exposed to BYMV infective aphids by releasing 10 to 12 pea aphids (Acyrthosiphon pisum) on each plant of the cultivar 'Diana' in the spreader rows. Viruliferous aphids were reared on caged BYMV-infected plants of the susceptible cv 'Diana' in the greenhouse. Aphids were released two to three times to ensure the rapid build-up of large aphid populations on the spreader rows.

In 1979 and 1980, faba bean selections were mechanically inoculated in both field and greenhouse tests by using an air-brush and a pressure of 276 kPa (40 psi) (Appendix 1).

In all greenhouse experiments, plants were inoculated mechanically at the four- to six-leaf stage and kept at 27° C until symptoms appeared.

Disease Assessment

Scales were developed to rate individual inoculated plants in the field and greenhouse separately for symptoms of mosaic, necrosis and stunting (Table 1). Evaluation of plants for resistance was made on the basis of host reactions to all symptom types. Plants with a 0 rating in all three symptom types were termed as highly resistant (HR) and plants with a maximum rating of 1 in any of the three types as 'moderately resistant' (MR). Plants with a rating of 0 or 1 were selected for further studies. Plants with a rating of 2 or more in any one type were considered susceptible and were not advanced. In all tests, selected symptomless plants were assayed for the presence of virus by inoculating susceptible 'Diana' plants in the greenhouse at 27° C with sap

Table 1. Scales for rating the symptom types of bean yellow mosaic virus in faba bean

| Mosaic | | Stunting | | Necrosis | |
|---|---|---|---|---|---|
| Scale | Symptoms | Scale | Symptoms | Scale | Symptoms |
| 0 | No symptoms | 0 | No stunting | 0 | No necrosis |
| 1 | Vein clearing or mild mottling | 1 | Some bushy top growth | 1 | A few necrotic spots on stem |
| 2 | Moderate mosaic | 2 | Bushy top growth with leaves reduced | 2 | Some necrotic streaks on stem |
| 3 | Severe mosaic with leaf bronzing | 3 | As 2 above plus plants severely stunted | 3 | Extensive necrotic streaks on stems; pod bronzing and/ or splitting |
| 4 | Severe mosaic with severe leaf distortion/ crinkling | | | 4 | Vascular necrosis, rapid death of plants |

from test plants extracted in 0.1 M phosphate buffer pH 7.4 (1:10, w/v). Host reactions were termed 'immune' if no virus was recovered.

# RESULTS

In general, selections used as susceptible controls developed severe symptoms of the disease in all the tests and years of testing. Other accessions including those selected previously for resistance expressed variable symptoms.

The results of the 1978 field tests showed that most of the selections and/or accessions tested were heterogeneous for different disease reactions. Symptomless plants were observed in very few selections, but plants with mild mosaic symptoms were observed in many selections (Appendix 2). The number of symptomless plants was greater in accessions that were previously selected as symptomless (2N425-3, 2N101-3, 2N4-1, 2N44-1) than in those not previously selected (2N425-1, 2N101-1, 2N101-2) or selected on the basis of mild symptoms (2N425-2, 2N101-4, 2N4-2, 2N44-2). BYMV was not detected in symptomless plants of eight of the 27 accessions assayed. Thus, plants immune to BYMV were identified in selections 2N43-2, 2N138-1, 2N85-1, 2N1-2, L5-22-1, 2N23-3, 2N101-3 and 2N425-1. In comparing results of the mechanically-inoculated trials with those of the aphid-inoculated trials, it was found that the majority of the accessions reacted similarly in both tests (Appendix 2). However, a few accessions such as 2N138-1, 2N40-3, 2N5-1, 2N101-3, L5-18-1 and UMFB15-2 showed a higher percentage of resistant plants in the aphid test than in the mechanically-inoculated test, whereas accessions such as 2N23-1, 2N40-2, 2N85-2, 2N4-1, 2N44-1 and 2N78-1 had more resistant plants in the mechanically-inoculated test

than in the aphid test. To avoid selecting plants that might have escaped infection in the aphid-inoculated tests, most of the 150 single or bulk plant resistant selections (0-1 rating) were made in the mechanical inoculation test.

Results of the 1979 field tests again showed considerable variation between and within accessions for disease reactions although many selections had a greater number of symptomless plants than in the previous year (Appendices 2 and 3). Thus, selections 2N43-2, 2N85-1, 2N101-1, 2N295-2 and 2N138-1 that had less than 6.9% symptomless plants in 1978, had 20.0, 35.7, 44.7, 36.4 and 77.8% symptomless plants, respectively, in 1979.

Twenty-five superior selections were retested in 1980. Ten of these along with 36 inbred lines selected in the greenhouse were retested in 1981 (Appendices 4 and 5). To illustrate the efficiency of selection, the data for 10 accessions that were reselected in each of the 4 years of testing (1978-1981) are summarized in Table 2 (Appendices 2-5). Only the highly resistant (HR) or moderately resistant (MR) plants of each accession converted to the percentage values, are presented. Progress in terms of obtaining uniformly-symptomless selections was more apparent in selections 2N23-3-2-1-1, 2N85-2-1-1-1, 2N138-1-1-1-1 and 2N295-2-1-1-1, although selections 2N101-1-1-1-2, L5-22-1-1-1-1 and 2N425-3-2-1-1 also had considerably more resistant (HR and MR) plants in 1980 and 1981 than in the previous 2 years. Selection 2N43-2-1-1-1 had fewer resistant plants in 1981 than in the previous 2 years. In reviewing the performance of accession 2N43-2, it was found that this accession had been more sensitive to infection by aphids than in the mechanical inoculation in 1978 (Appendix 2). This may explain the sharp decline in number of resistant plants in

Table 2. Effectiveness of selection for resistance to 'mosaic' strain of bean yellow mosaic virus for 10 faba bean accessions evaluated in the field from 1978 to 1981

| Accession | Disease reaction† | Percent Plants | | | |
|---|---|---|---|---|---|
| | | 1978§ | 1979†† | 1980 | 1981 |
| 2N23-3-2-1-1 | HR | 6.7 | 2.5 | 66.7 | 73.3 |
| | MR | 26.7 | 37.5 | 33.3 | 20.0 |
| 2N43-2-1-1-1 | HR | 4.9 | 20.0 | 55.6 | 0.0 |
| | MR | 7.3 | 52.0 | 27.8 | 36.4 |
| 2N85-2-1-1-1 | HR | 10.8 | 35.7 | 57.1 | 61.5 |
| | MR | 32.4 | 28.6 | 28.6 | 23.1 |
| 2N101-1-1-1-2 | HR | 2.6 | 33.3 | 85.7 | 76.9 |
| | MR | 20.5 | 16.7 | 14.3 | 15.4 |
| 2N138-1-1-1-1 | HR | 6.9 | 77.7 | 94.1 | 100.0 |
| | MR | 6.9 | 5.6 | 5.9 | 0.0 |
| L5-22-1-1-1-1 | HR | 6.7 | 37.9 | 30.4 | 35.3 |
| | MR | 20.0 | 24.2 | 52.2 | 47.1 |
| 2N295-2-1-1-1 | HR | 0.0 | 34.6 | 25.0 | 85.7 |
| | MR | 57.2 | 42.3 | 68.8 | 14.3 |
| 2N425-3-2-1-1 | HR | 0.0 | 16.7 | 16.7 | 61.5 |
| | MR | 17.7 | 33.3 | 33.3 | 30.8 |
| UMFB13-1GH-1 | HR | 0.0 | 4.0 | 0.0 | 5.9 |
| | MR | 0.0 | 0.0 | 0.0 | 5.9 |
| Diana | HR | 0.0 | 14.3 | 0.0 | 10.0 |
| | MR | 0.0 | 21.4 | 0.0 | 10.0 |

†HR = Highly resistant with a maximum of 0 rating and MR = Moderately resistant with a maximum of 1 rating in any of the symptom types.

§Based on three replications.

††Based on two replications.

this accession in 1981 when the accessions were exposed to viruliferous aphids than when mechanically inoculated.

By 1981, all the plants in three selections from accession 2N138-1 were symptomless (Table 2; Appendix 4). In addition, selections 2N23-3-2-1-1, 2N101-1-1-1-2, L5-22-1-1-1-1 and 2N5-2-1-1-1 had more than 80% resistant plants in 1981 as compared to less than 35% in the year 1978. The majority of the selections, however, were still heterogeneous after 4 years of selection (Appendices 2-5). Differential response of accessions to cross-pollination and aphid infestation might account for the differences in obtaining uniformly resistant selections.

Virus assays of the symptomless plants observed in 1979 through 1981 revealed that most of the plants had latent infections to BYMV. However, symptomless selections from 2N138-1, 2N4-1, 2N23-3-2-1-1 and 2N425-3-2-1-1 were virus-free and considered immune.

Results of tests in the greenhouse of 125 single and bulk plant selections from the field along with the results of the 55 superior selections tested in 1979 are summarized in Table 3. The results revealed that 35 days after inoculation with the 'mosaic' strain, three accessions, 2N138-1GH-3, 2N295-2GH-1 and 2N23-2GH-3, remained symptomless in both years and in 1979, three additional selections, L5-22-1GH-1-1, UMFB15-1GH-1-1 and L5-42-1GH-1, were also symptomless. Several additional selections had either all plants moderately resistant or were heterogeneous for disease reactions (Table 3).

Eighteen superior selections from the field testing program and 22 inbred lines from the greenhouse program were evaluated against the 'necrotic' strain in the field and greenhouse, respectively. Only selections with symptomless plants or plants with mild symptoms are listed in

Table 3. Faba bean selections with resistance to the 'mosaic' strain of bean yellow mosaic virus in the greenhouse at 27° C

| Disease reaction | Faba Bean Selections* | |
|---|---|---|
| | 1978 | 1979 |
| All plants resistant† | 2N138-1GH-3, 2N295-2GH-1, 2N23-2GH-3 | 2N138-1GH-3-1, 2N295-2GH-1-5, 2N23-2GH-3-2, L5-22-1GH-1-1, UMFB15-1GH-1-1, L5-42-1GH-1 |
| All plants moderately resistant§ | 2N23-3GH-4, 2N23-1GH-1, L5-22-1GH-1, UMFB15-1GH-1, 2N295-1GH-1, 7844-1GH-1 | 2N295-1GH-1-4, 2N425-3GH-2-1, 2N23-1GH-1-1, UMFB13-1GH-1-3, 2N101-2GH-2-3 |
| Heterogeneous with resistant and susceptible plants | 2N23-3GH-2, 2N85-2GH-1, 2N138-1GH-1, 2N425-3GH-2, 2N101-2GH-2, UMFB13-1GH-1, L5-13-2GH-1, 2N21-1GH-3, L5-22-2GH-1, 2N85-2GH-3 | 2N23-3GH-2-1, 2N23-1GH-1-1, UMFB13-1GH-1-6, 2N425-3GH-2-3, 2N101-2GH-2-2, L5-13-2GH-1-3, 2N138-1GH-1-4, Diana-1, 2N85-2GH-1-1, 2N236-1GH-1, C104/308/73-1GH-1, NA52A-1GH-1 |

*Five to 10 plants per selection were tested.

†All plants had a disease rating of 0 (symptomless), 28 days after inoculation.

§All plants in the lines had a maximum disease rating of 1 in any of the three symptom types, mosaic, stunting and necrosis, 28 days after inoculation.

Table 4. Selections 2N138-1-1-1 and 2N138-1GH-3-5 were immune to BYMV in the field and greenhouse, respectively, whereas another selection, 2N138-1-1-2, had only mild symptoms in the field (Table 4). A large number of lines were again heterogeneous with only a few resistant plants. Some selections from accessions 2N23, 2N295, L5-22 and 2N85 with resistance to the 'mosaic' strain were found to be highly susceptible to the 'necrotic' strain in the field and greenhouse tests.

Table 4. Faba bean selections with resistance to the 'necrotic' strain of bean yellow mosaic virus in the field and greenhouse

| Disease reaction | Faba Bean Selections* | |
|---|---|---|
| | Field | Greenhouse |
| All plants resistant† | 2N138-1-1-1 | 2N138-1GH-3-5 |
| All plants moderately resistant§ | 2N138-1-1-2 | NIL |
| Heterogeneous with resistant and susceptible plants | 2N1-2-1-1, 2N295-2-1-1, UMFB13-1-1-2, 2N43-2-1-1, L5-22-1-1-1, 2N101-1-1-1, 2N138-1-2-1 | 2N23-3GH-1-1, 2N101-2GH-8-1, 78168-1GH-1, U-11-1GH-1, UMFB15-1GH-1-2, C104/308/73-1GH-1, NA52A-1GH-1 |

*Five to 10 plants per selection were tested.

†All plants had a disease rating of 0 (symptomless), 28 days after inoculation.

§All plants in the lines had a maximum disease rating of 1 in any of the three symptom types, mosaic, stunting and necrosis, 28 days after inoculation.

# DISCUSSION

The procedure for testing and selecting single plants for resistance (symptomless or mild mosaic) to BYMV was effective in the field and highly effective in the greenhouse where plants were self-pollinated. Selection was also effective to obtain genotypes developing severe mosaic and stunting but no stem or vascular necrosis in response to BYMV infection. High selection efficiency occurred in accessions 2N138-1, 2N23-3, 2N85-2 and 2N295-2 that resulted in greater uniformity in obtaining symptomless plants over 4 years of testing. Four other accessions (2N43-2, 2N101-1, L5-22-1 and 2N425-3) tested for 4 years did not result in a progressive increase in number of symptomless plants, although the number of resistant (symptomless and mild mosaic) plants increased in each succeeding year. Open-pollination or genetic linkage might explain the lack of progress in these accessions. With the exception of selections from 2N138-1, selections were still heterogeneous at the end of 4 years. In comparison, 2 years of greenhouse selection resulted in 11 lines that were uniformly resistant to the 'mosaic' strain.

Although the emphasis was to identify lines resistant to the 'mosaic' strain, the selected genotypes were also inoculated with the less common but more severe 'necrotic' strain of BYMV. Only the selections from accession 2N138 were found to be uniformly resistant to this strain. A number of additional selections were heterogeneous but included a few resistant plants and therefore, provide opportunity for further selection and improvement. The fact that one selection was resistant to both strains of BYMV, whereas other selections were resistant to the 'mosaic' strain

and not to the 'necrotic' strain would seem to indicate that resistance to each strain is controlled by different factor(s).

The relative stability of resistance in selected accessions over 4 years of field testing suggests that the resistance is of practical value in breeding faba beans for resistance to BYMV.

## 3.2 Inheritance of Resistance to Bean Yellow Mosaic Virus in *Vicia faba*


### ABSTRACT

An immune and a highly resistant faba bean inbred line were crossed reciprocally with each of two susceptible inbred lines to study the inheritance of resistance to two strains of bean yellow mosaic virus (BYMV). The data from testing $F_1$, $F_2$ and $F_3$ generations in the greenhouse at 27° C and some $F_2$ generations in the field, supported the hypothesis that resistance in line 2N23-2GH-3-2 to the 'mosaic' strain and immunity in line 2N138-1GH-3-5 to the 'mosaic' and 'necrotic' strains of BYMV were each conditioned by two recessive genes. Highly susceptible $F_2$ plants developed symptoms at 14 days and moderately susceptible $F_2$ plants at 21 or 28 days after inoculation at 27° C. The $F_3$ progenies from moderately susceptible $F_2$ plants segregated to fit a 3 susceptible:1 resistant ratio indicating that the delay in symptom expression was due to the heterozygous condition of a single dominant gene. One of the two genes for susceptibility in line UMFB13-1GH-1-3 was masked in the greenhouse and expressed only in the field.

## INTRODUCTION

Among the viruses affecting faba beans (Vicia faba L.), bean yellow mosaic virus (BYMV) is common and widely distributed in many regions of the world (Kaiser et al., 1968; El-Attar et al., 1971; Bos, 1981). The virus has been frequently observed in faba beans grown in the province of Manitoba (Bernier, 1975) and two strains, 'mosaic' and 'necrotic', have been reported (Frowd and Bernier, 1977). Reactions of BYMV in various faba bean cultivars included symptomless infection, mild or severe mosaic with or without severe stunting or tissue necrosis, depending upon host genotype, virus strain and the environment.

In Manitoba, BYMV is transmitted by viruliferous pea aphids (Acyrthosiphon pisum Harris) and yield losses as high as 96.3% have occurred in faba beans as a result of early infections (Frowd and Bernier, 1977). Breeding resistant cultivars can be one of the most effective methods of controlling virus diseases, therefore, attempts were made to identify faba bean plants with resistance to BYMV (Thesis Section 3.1). During 1976 through 1980, some 360 accessions were screened for resistance to the 'mosaic' strain of BYMV in the field. Eighteen superior selections from the field and 22 from the greenhouse were further evaluated for reaction to the 'necrotic' strain in the field and greenhouse, respectively. Fifteen selections from the field and 11 from the greenhouse tests had most of the plants resistant to the 'mosaic' strain, whereas nine selections in the field and eight in the greenhouse also had a few plants resistant to the 'necrotic' strain (Thesis Section 3.1). The resistant selections included plants that were either symptomless with latent virus

infections or were free of both symptoms and virus (immune) on the basis of virus assays. Faba beans partially outcross in the field (Toynbee-Clarke, 1974; Poulsen, 1975), therefore, field selections required three cycles of testing and selfing in the greenhouse to attain homozygosity prior to being used in the inheritance of resistance studies reported herein.

## MATERIALS AND METHODS

The reactions of the four inbred lines used as parents are described in Table 5. One immune and one highly resistant faba bean inbred line were reciprocally crossed to each of two susceptible faba bean inbred lines. The parents, $F_1$, $F_2$ and $F_3$ generations, were tested in the greenhouse at a minimum temperature of 27° C. Two $F_2$ generations from crosses involving UMFB13 as a susceptible parent were also tested in the field. Some $F_1$ plants were tested against the two virus strains separately, the rest were retained for $F_2$ seed production. Only the $F_3$ progenies from resistant or moderately susceptible $F_2$ plants were tested since no progenies were obtained from highly susceptible $F_2$ plants. In the greenhouse, faba bean plants were grown in clay pots 12-cm in diameter or in 60 x 30 x 10-cm plastic trays containing 2:1:1 (v/v) mixture of soil, sand and peat moss. Fertilizer and/or systemic insecticides were applied as necessary to ensure good growth and freedom from aphids and mites. In the field, plants were grown in rows 1.5-m long and 60-cm apart, each containing 15-20 plants.

Two BYMV strains, 'mosaic' and 'necrotic', reported by Frowd and Bernier (1977), were used in the present studies and maintained and propagated in a susceptible faba bean cultivar 'Diana'. The inoculum was prepared by grinding young infected tissue in 0.1 M phosphate buffer pH 7.4 (1:10, w/v). The sap was clarified by straining it through two to four layers of cheesecloth. Before inoculation carborundum (600 mesh) was mixed at 2% (w/v) with the inoculum. Plants with four to six fully expanded leaves were pressure-inoculated by an air brush using 276 kPa

Table 5. Reaction of parental faba bean inbred lines to 'mosaic' and 'necrotic' strains of bean yellow mosaic virus

| Inbred line | 'Mosaic' Strain | | 'Necrotic' Strain | |
|---|---|---|---|---|
| | Reaction class | Symptoms | Reaction class | Symptoms |
| 2N138-1GH-3-5 | Immune | No symptoms, no virus recovered from young tissues | Immune | No symptoms, no virus recovered |
| 2N23-2GH-3-2 | Highly resistant | No symptoms but virus recovered | Susceptible | Severe mosaic but no vascular necrosis |
| UMFB13-1GH-1-3 | Susceptible | Mosaic, leaf curling and severe stunting, very little or no stem necrosis | Susceptible | Vascular necrosis and rapid death of plants |
| DIANA-1GH-2-3 | Susceptible | Severe mosaic, stunting and stem necrosis | Susceptible | Vascular necrosis and rapid death of plants |

(40 psi) pressure as described previously (Thesis Section 3.1). Plants were sprayed for 5 seconds and washed with tap water immediately after inoculation. Twenty to 25 plants of the susceptible cultivar 'Diana' were inoculated along with the test plants to verify the infectivity of the inoculum. Plant reactions to the 'mosaic' strain were recorded 14, 21 and 28 days and to the 'necrotic' strain, 7, 14 and 28 days after inoculation. Plants that showed clear symptoms 14 days after inoculation were considered highly susceptible; plants that showed symptoms 21 or 28 days after inoculation were considered moderately susceptible and plants that remained free of symptoms, resistant.

## RESULTS AND DISCUSSION

The parental inbred lines developed symptoms described in Table 5 (Thesis Section 3.1). Plants of the susceptible inbred lines developed symptoms 14 days after inoculation at 27° C in the greenhouse and in 14-21 days in the field. Plants of the resistant inbred lines were free of symptoms 28 days after inoculation.

The data on segregation of $F_1$, $F_2$ and $F_3$ generations into resistant and susceptible plants when tested against the 'mosaic' strain are presented in Table 6. All $F_1$ plants were classified as susceptible, indicating that resistance to BYMV is conditioned by a recessive factor or factors. Symptoms were visible in the majority of $F_1$ plants on the 14th day of inoculation, however, some plants that had UMFB13-1GH-1-3 as the susceptible parent expressed symptoms during the next 14 days.

A good fit for a 15:1 ratio of susceptible:resistant plants was observed in the $F_2$ generations from crosses having Diana-1GH-2-3 as the susceptible parent. The $F_2$ plants from crosses involving UMFB13-1GH-1-3 as the susceptible parent segregated into 3 susceptible:1 resistant ratio in the greenhouse (Table 6) and 15 susceptible:1 resistant ratio in the field (Table 7). Resistance or susceptibility thus appears to be controlled by two genes while one of the two genes for susceptibility from UMFB-1GH-1-3 either was not expressed in the greenhouse or was masked by a modifier gene(s), activated under the greenhouse conditions only.

A majority of the $F_2$ plants developed symptoms 14 days after inoculation (highly susceptible) while about one-third took 7-14 days longer

Table 6. Segregation of $F_1$, $F_2$ and $F_3$ generations in response to infection by the 'mosaic' strain of bean yellow mosaic virus at 27° C

| Population | Plants tested | Disease Reaction S* | Disease Reaction R† | Exp. ratio | P- for $X^2$ |
|---|---|---|---|---|---|
| 2N138-1GH-3-5 X Diana-1GH-2-3 | | | | | |
| $F_1$ | 35 | 35 | 0 | 1:0 | |
| $F_2$ | 493 | 464 | 29 | 15:1 | .75-.90 |
| $F_3$ from R $F_2$§ | 50 | 2 | 48 | 0:1 | |
| $F_3$ from MS $F_2$¶ | 60 | 44 | 16 | 3:1 | .75-.90 |
| 2N23-2GH-3-2 X Diana-1GH-2-3 | | | | | |
| $F_1$ | 80 | 80 | 0 | 1:0 | |
| $F_2$ | 509 | 469 | 40 | 15:1 | .10-.25 |
| $F_3$ from R $F_2$§ | 37 | 4 | 33 | 0:1 | |
| $F_3$ from MS $F_2$¶ | 91 | 75 | 26 | 3:1 | .90-.95 |
| 2N138-1GH-3-5 X UMFB13-1GH-1-3 | | | | | |
| $F_1$ | 70 | 70 | 0 | 1:0 | |
| $F_2$ | 305 | 233 | 72 | 3:1 | .50-.75 |
| $F_3$ from R $F_2$§ | 36 | 6 | 30 | 0:1 | |
| 2N23-2GH-3-2 X UMFB13-1GH-1-3 | | | | | |
| $F_1$ | 48 | 48 | 0 | 1:0 | |
| $F_2$ | 381 | 280 | 101 | 3:1 | .50-.75 |
| $F_3$ from R $F_2$§ | 28 | 8 | 20 | 0:1 | |

*S = Susceptible; Plants with symptoms 28 days after inoculation.

†R = Resistant; Plants free of symptoms 28 days after inoculation.

§$F_3$ population derived from resistant (R) $F_2$ plants.

¶$F_3$ population derived from moderately susceptible (MS) $F_2$ plants that expressed symptoms 21 or 28 days after inoculation.

Table 7. Segregation of $F_2$ generations in response to infection by the 'mosaic' strain of bean yellow mosaic virus in the field.

| Cross | Plants tested | Reaction | | Exp. ratio | P for $X^2$ |
|---|---|---|---|---|---|
| | | S* | R† | | |
| 2N138-1GH-3-5 X UMFB13-1GH-1-3 | 207 | 195 | 12 | 15:1 | .75-.90 |
| 2N23-2GH-3-2 X UMFB13-1GH-1-3 | 458 | 420 | 38 | 15:1 | .05-.10 |

*S = Susceptible; Plants with symptoms 28 days after inoculation.

†R = Resistant; Plants without symptoms 28 days after inoculation.

to show symptoms (moderately susceptible). When the $F_2$ progenies were classified into highly susceptible, moderately susceptible and resistant plants, the $X^2$ test for each of four $F_2$ generations fit best a 11:4:1 ratio ($P \geq .50$), supporting again the hypothesis that resistance is dependent on the homozygous condition of two recessive genes.

Results of $F_3$ progeny tests showed that most of the progenies from resistant $F_2$ plants were resistant, whereas $F_3$ progenies from moderately susceptible (MS) $F_2$ plants segregated into 3 susceptible:1 resistant ratio (Table 6). A single dominant gene in the heterozygous condition is hypothesized to cause delay of symptoms from 7-14 days in the moderately susceptible $F_2$ plants.

The classification of plants into susceptible or resistant groups based on their reactions on the 28th day of inoculation appeared logical since symptoms developing later would have little or no effect on plant growth. However, incorrect classification of a few susceptible plants into the resistant category apparently occurred because their $F_3$ progenies segregated for two types of reactions. Uneven temperature in the greenhouse and modifying gene action could also account for lack of symptoms in a few $F_2$ plants. Because susceptibility is dominant, the low number of susceptible plants in the $F_3$ progenies from resistant $F_2$ plants was of little consequence since most were resistant.

The $F_2$ generations from crosses between the resistant (2N138-1GH-3-5) and susceptible (Diana-1GH-2-3) plants segregated into 15 susceptible: 1 resistant ratio against the 'necrotic' strain (Table 8) indicating that, again, two genes were involved. Most of the $F_2$ plants from crosses involving two susceptible plants, 2N23-2GH-3-2 and Diana-1GH-2-3, were susceptible. Uneven temperature and modifying gene action might again

Table 8. Segregation of $F_2$ generations in response to infection by the 'necrotic' strain of bean yellow mosaic virus at 27° C

| Cross | Plants tested | Reaction | | Exp. ratio | P for $X^2$ |
|---|---|---|---|---|---|
| | | S* | R† | | |
| 2N138-1GH-3-5 X Diana-1GH-2-3 | 252 | 235 | 17 | 15:1 | .75-.90 |
| 2N23-1GH-3-2 X Diana-1GH-2-3 | 112 | 109 | 3 | 1:0 | .90 |

*S = Susceptible; Plants with symptoms 28 days after inoculation.

†R = Resistant; Plants without symptoms 28 days after inoculation.

have influenced the expression of symptoms in a few susceptible plants that appeared to be resistant.

The fact that line 2N23-2GH-3-2 is susceptible and line 2N138-1GH-3-5 resistant to the 'necrotic' strain, whereas both inbred lines are resistant to the 'mosaic' strain suggests that the two genes for resistance to the 'mosaic' strain are not the same as the two genes that condition resistance to the 'necrotic' strain. The presence of additional gene(s) for resistance in line 2N138-1GH-3-5 and susceptibility in line 2N23-2GH-3-2 to 'necrotic' strain is thus indicated. Moreover, since line 2N138-1GH-3-5 is immune and line 2N23-2GH-3-2 symptomless to the 'mosaic' strain, the two genes governing resistance in the two lines to the 'mosaic' strain may again be different. Intercrossing the two resistant lines may provide genetic ratios to determine their genotypes.

Results of this study are in agreement with those of Baggett (1956) and Baggett and Frazier (1957) who reported two and possibly three recessive genes governing resistance in Phaseolus coccineus and a P. vulgaris variety, Northern U.I. 31 to two BYMV strains. Effects of some modifier genes on type and severity of symptoms were also noted by these authors. Yen and Fry (1956), Johnson and Hagedorn (1958), Schroeder and Provvidenti (1962) and Barton et al. (1964) also identified a single recessive gene 'mo' that conferred resistance to different BYMV isolates in peas.

Studies by Yen and Fry (1956) and Schroeder et al. (1960) revealed that symptom development in peas in response to infection by BYMV is influenced by temperature. At a temperature of 27° C the susceptible plants heterozygous for a dominant gene for susceptibility (Momo) developed symptoms as quickly as the homozygous plants (MoMo). Symptoms were delayed or were masked when inoculated heterozygous plants were incubated at a

lower temperature (18° C). In the present study, not all susceptible faba bean plants developed symptoms after 14 days at 27° C and some required 7-14 days longer to develop symptoms. It appeared that in addition to temperature, genetic make-up and heterozygosity determined incubation time required for the expression of symptoms. Thus, a plant heterozygous for both genes expressed symptoms within 14 days like a completely homozygous plant. Plant heterozygous with only one dominant gene would take 7-14 days longer to develop symptoms, depending possibly on which of the two dominant genes was heterozygous. The delay in symptom expression might also be due to additive gene action by the three recessive alleles.

The investigations reported herein reveal the heritable nature of resistance of faba bean lines to the two BYMV strains from Manitoba. Although further studies are necessary to fully understand the genetic make-up of the two resistant lines, the results provided strong evidence that the resistance of 2N23-2GH-3-2 to the 'mosaic' strain and immunity of 2N138-1GH-3-5 to both virus strains are simply inherited and each conditioned by two recessive genes. These findings may prove useful in the development of faba beans having a broader base of genetic resistance to BYMV.

## 3.3 Effect of Temperature and Inoculum Concentration on Reactions of Inbred Lines of Faba Bean (*Vicia faba*) to Bean Yellow Mosaic Virus


### ABSTRACT

The effect of temperature and inoculum concentration of bean yellow mosaic virus (BYMV) on host reaction was studied in three faba bean inbred lines, 2N138-1GH-3-5, 2N23-2GH-3-2 and Diana-1GH-2-3, identified previously as immune, symptomless and susceptible to the 'mosaic' strain and immune, susceptible and susceptible to the 'necrotic' strain, respectively. Temperature variations from 17-21° C to 23-27° C had little effect on the time required for symptoms to appear, however, symptoms were more severe at the higher temperature. Plants of susceptible line Diana-1GH-2-3 were not infected when inoculated with an inoculum concentration of 1:1000 (w/v of infected leaves to distilled water) or when two pea aphids (*Acyrthosiphon pisum* Harris) were used to transmit the 'mosaic' strain. Line 2N23-2GH-3-2 was infected but remained symptomless when higher inoculum concentrations exceeded 1:1000 (w/v infected leaves : distilled water) or when four and eight viruliferous aphids per plant were used. The normally immune line 2N138-1GH-3-5 was infected in the greenhouse when four or eight viruliferous aphids per plant were used or when epidermal strips were removed from leaves before plants were mechanically inoculated. However, inbred lines and field selections from 2N138 were immune when viruliferous aphids were used to inoculate the plots in the field.

## INTRODUCTION

Symptom development as a result of viral infections, particularly in heterozygous susceptible plants, may be influenced by the temperature at which plants are incubated after inoculation. Pea plants heterozygous for a gene (Momo) expressed symptoms when inoculated with bean yellow mosaic virus (BYMV) and incubated at 27° C, whereas the symptoms were masked when inoculated plants were kept at 18° C or lower (Schroeder et al., 1960; Schroeder et al., 1966). Phenotypic changes as a result of changes in temperature regimes during incubation were also noticed by Pelham (1970) in tomato plants heterozygous (Tm-2/+) for a resistance gene (Tm-2) to tobacco mosaic virus (TMV). Pilowsky et al. (1981), on the other hand, demonstrated that differential amounts of TMV introduced into the tomato plants produced different reactions in heterozygous versus homozygous plants incubated at the same temperature (30-31° C).

In previous studies, three main host reactions were recognized in faba bean (Vicia faba L.) germplasm inoculated with BYMV; immune, symptomless but infected and severe symptoms of mosaic and necrosis (Thesis Section 3.1). Plants that developed each type of reaction were inbred for three generations in the greenhouse so that they were homozygous. In the field, BYMV is transmitted to faba bean primarily by the pea aphids, Acyrthosiphon pisum (Harris) (Frowd and Bernier, 1977) and plants may be repeatedly inoculated with the virus, depending upon the size of aphid populations and their movement. In general, stylet-borne viruses are readily transmissible by aphids (D'Arcy and Nault, 1982) and in many cases, one or two viruliferous aphids may be sufficient to infect

susceptible plants (Sylvester, 1952; Bradley and Rideout, 1953; Watson and Roberts, 1939). However, the effect of repeated infections by viruliferous aphids on the stability of immune or symptomless reactions of faba bean inbred lines is unknown. Large temperature variations in the field might also alter host reactions to BYMV (Schroeder and Provvidenti, 1964).

The objectives of this study were to determine the effects of temperature and varying concentrations of inoculum on the reactions of three inbred lines of faba bean to BYMV.

## MATERIALS AND METHODS

The faba bean inbred lines 2N138-1GH-3-5, 2N23-2GH-3-2 and Diana-1GH-2-3, identified as immune, symptomless and susceptible, respectively, to the 'mosaic' strain of BYMV, were used in this study (Table 9). The susceptible line developed severe mosaic, stunting and stem necrosis. Two BYMV strains, 'mosaic' and 'necrotic', identified previously by Frowd and Bernier (1977) were used. The 'mosaic' strain carried vein clearing, mottling, mosaic and bronzing of leaves and eventually severe stunting, stem necrosis and premature pod splitting in more susceptible plants. The symptoms caused by the 'necrotic' strain ranged from mosaic with numerous systemic necrotic spots to tip or vascular necrosis and rapid death of the more susceptible plants. Both virus strains were maintained and propagated in the susceptible faba bean cultivar 'Diana' in the greenhouse. Inoculum was prepared as described previously (Thesis Section 3.1) and unless otherwise stated, four young leaves of 3-week-old faba bean plants were mechanically inoculated using a pipe cleaner dipped in sap from infected leaves, diluted 1:10 (w/v) with 0.1 M phosphate buffer pH 7.4. Each dip was adequate to inoculate two leaves.

### Effect of Temperature on Symptom Development

Eight to 10 plants of each inbred line were mechanically inoculated with each of two BYMV strains in separate tests and incubated at two day and night temperature regimes, 21-17$^{o}$ C and 27-23$^{o}$ C. Symptoms were recorded and leaf samples of each plant were collected for virus assays

Table 9. Reaction of faba bean inbred lines to the 'mosaic' strain of bean yellow mosaic virus in field (1981) and greenhouse at 27° C (1979)

| Line | Field* | | | | Greenhouse§ | | | |
|---|---|---|---|---|---|---|---|---|
| | Plants tested | Plants with symptoms | Virus recovered** | Overall reaction | Plants tested | Plants with symptoms | Virus recovered** | Overall reaction |
| 2N138-1GH-3-5 | 31 | 0 | - | Immune | 10 | 0 | - | Immune |
| 2N23-2GH-3-2 | 32 | 7 | + | Latent/ mild symptoms | 15 | 0 | + | Latent |
| DIANA-1GH-2-3§§ | 70 | 59 | + | Susceptible | 12 | 12 | + | Susceptible |

*Tested with viruliferous pea aphids.

§Tested with mechanical inoculation.

**Only selected symptomless plants were assayed; - = virus not recovered, + = virus recovered.

§§In field, commercial seed of the cultivar Diana was used.

2, 4, 6, 8, 10, 12, 19, 26 and 33 days after inoculation. Two methods of assay were compared in order to obtain an effective method of assaying virus from a large number of samples each weighing less than 2.0 g. Nine species and cultivars reported to produce local lesions in response to BYMV infection were tested. However, none consistently produced numbers of local lesions proportional to the virus concentration (Appendix 6). The production and the number of local lesions appeared to be greatly affected by the plant growth stage, environmental factors and uniformity in the cultivar used.

The enzyme linked immunosorbant assay (ELISA) recently introduced by Clark and Adams (1977) has been used to assay viruses in many plant species (Converse, 1978; Hamilton and Nicholas, 1978; Lister, 1978; Bar-Joseph et al., 1979; Stein et al., 1979; Adams et al., 1980; Clarke, 1980; Banttari, 1981; Banttari and Franc, 1981; Hoy et al., 1981; Lommel et al., 1981). However, attempts made in the present study to use the standard ELISA technique of Clark and Adams (1977) to assay BYMV in faba bean were unsuccessful because of excessive background reactions due to non-specific host proteins in the antisera. No clear distinctions could be seen between the absorbance values of virus specific and non-specific reactions. All the modifications attempted were also not successful (Appendix 7). Therefore, a susceptible faba bean cultivar 'Diana' that developed a systemic infection and mosaic was used to assay virus in inoculated test plants. Since the method required the use of many plants for each of the dilutions tested, only selected samples were quantitatively assessed for virus titres. Generally, 30 'Diana' plants were used for each dilution.

Effect of Inoculum Concentration on Symptom Development

The effect of inoculum concentration on the three inbred faba bean lines was assessed with the 'mosaic' strain by comparing three dilutions of the inoculum, by varying the number of leaves mechanically inoculated on each plant or by inoculating plants with varying numbers of aphids. Inoculum was prepared by triturating young infected leaves in 0.1 M phosphate buffer pH 7.4 (1:5, w/v) and diluting to 1:10, 1:100 and 1:1000 with distilled water. Eight to 10 plants per treatment were used in tests where plants were inoculated mechanically. For aphid tests, young healthy pea aphids (Acyrthosiphon pisum) were starved for 3 hours, released for 10 minutes on virus-infected leaves and then two, four or eight aphids were transferred and allowed to feed for 24 hours on each of the five plants used per treatment. Aphids were then killed with a spray of the insecticide Pirimor. Symptoms were recorded and leaf samples from inoculated plants were collected and assayed for virus as described for temperature studies.

In all experiments, five to 10 uninoculated susceptible plants were incubated as controls along with the inoculated plants and were assayed for virus periodically.

# RESULTS

At the termination of the experiments, none of the control plants had symptoms typical of BYMV and virus was not recovered from them.

## Effect of Temperature on Symptom Development

Results summarized in Table 10 show that inbred line 2N138-1GH-3-5 was immune to infection by both virus strains at both temperature regimes, confirming previous results (Thesis Section 3.1). Inbred line 2N23-2GH-3-2 was symptomless but with latent infection when inoculated with the mosaic strain and developed systemic necrotic spots followed by severe mosaic and stunting to the 'necrotic' strain 12 days after inoculation at both temperature regimes. Inbred line Diana-1GH-2-3, on the other hand, developed systemic symptoms to both virus strains. At both temperature regimes, symptoms caused by the 'mosaic' strain appeared 4 days later than symptoms caused by the 'necrotic' strain. The period of time for virus to become detectable, however, did not differ appreciably between the resistant and susceptible inbred lines (Table 10). In both the symptomless (2N23-2GH-3-2) and susceptible (Diana-1GH-2-3) inbreds, the virus was detected 6-8 days after inoculation. Inbred lines 2N23-2GH-3-2 and Diana-1GH-2-3 were both susceptible to the 'necrotic' strain and, in general, symptoms were more severe at higher than at lower temperatures. Virus concentration of selected plants of each inbred line inoculated at 27-23$^{o}$ C regime was estimated by dilution-infectivity curves (Fig. 1). Virus concentration could be effectively compared at 1:100 or 1:1000 dilution of infective leaf extracts since at lower dilutions the differences

Table 10. Effect of temperature on symptoms caused by two strains of bean yellow mosaic virus in faba bean inbred lines

| Temperature regime | Line | 'Mosaic' Strain | | | 'Necrotic' Strain | | |
|---|---|---|---|---|---|---|---|
| | | Reaction* | D.S.† | D.V.§ | Reaction* | D.S.† | D.V.§ |
| 21 - 17° C | | | | | | | |
| | 2N138-1GH-3-5 | I | - | - | I | - | - |
| | 2N23-2GH-3-2 | L | - | 8 | S | 12 | 10 |
| | DIANA-1GH-2-3 | S | 10 | 8 | S | 6 | 6 |
| 27 - 23° C | | | | | | | |
| | 2N138-1GH-3-5 | I | - | - | I | - | - |
| | 2N23-2GH-3-2 | L | - | 8 | S | 12 | 8 |
| | DIANA-1GH-2-3 | S | 10 | 6 | S | 6 | 6 |

*I = Immune; L = Latent; S = Susceptible.

†D.S. = Days taken to express symptoms; - = symptoms not expressed up to 33 days after inoculation.

§D.V. = Days taken for virus to become detectable; - = virus not recovered up to 33 days after inoculation.

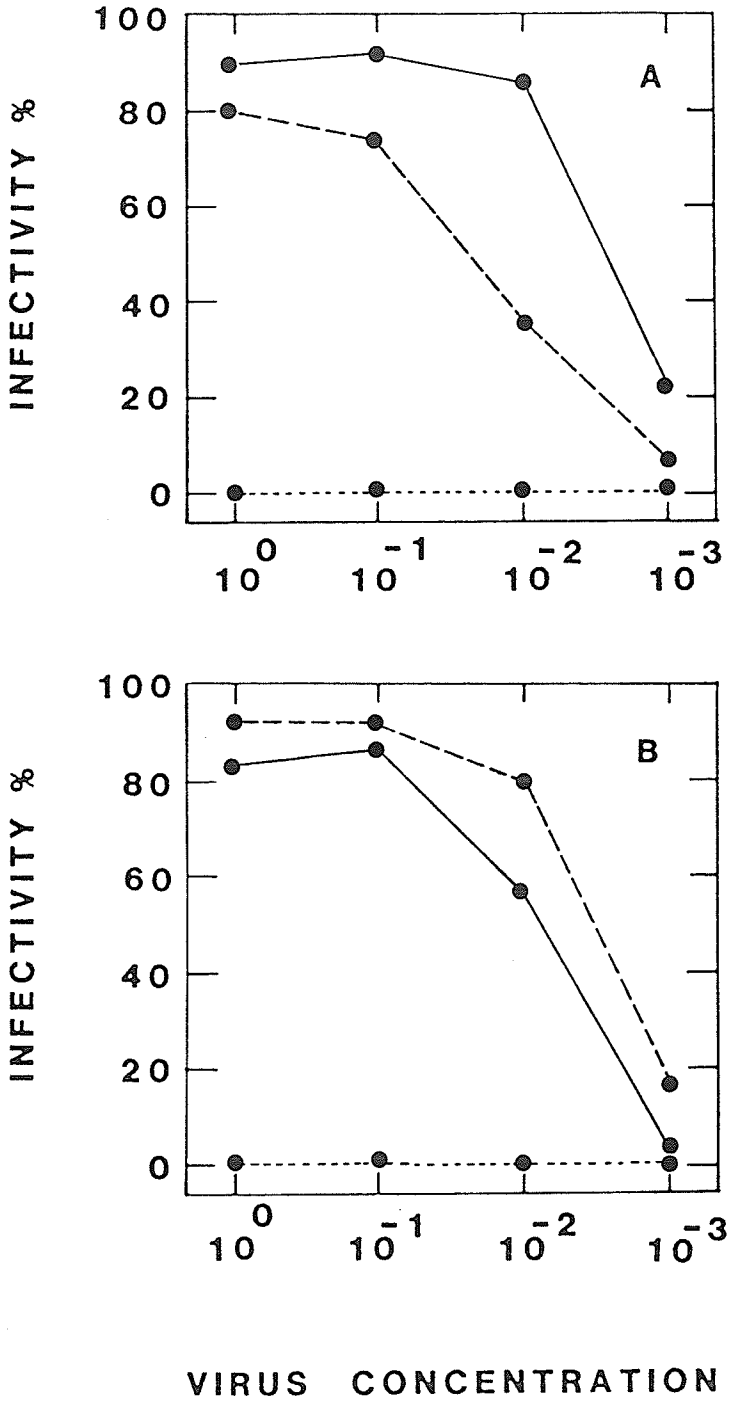


Fig. 1. Dilution-infectivity curves of leaf extracts from lines 2N138-1GH-3-5 (----), 2N23-2GH-3-2 (– – –) and Diana-1GH-2-3 (——), 12 days after inoculation with (A) 'mosaic' strain and (B) 'necrotic' strain of bean yellow mosaic virus, based on 30 systemic indicator plants of faba bean cv 'Diana'.

in virus infectivity (V.I.) were not readily distinguished (Fig. 1). At 1:100 dilution, leaf extracts from 'mosaic'-infected 2N23-2GH-3-2 plants had only 36.7% infectivity as compared to 86.7% in leaf extracts from 'mosaic'-infected Diana-1GH-2-3 plants. Line 2N138-1GH-3-5 remained free of virus irrespective of the dilutions tested. The symptomless nature of line 2N23-2GH-3-2 to 'mosaic' strain may, therefore, be attributed to a reduced rate of viral multiplication. On the other hand, line 2N23-2GH-3-2 which is susceptible to the 'necrotic' strain, had more viral infectivity at all dilutions than line Diana-1GH-2-3, although symptoms were more severe in line Diana-1GH-2-3 (vascular necrosis and host collapse) than in line 2N23-2GH-3-2 (systemic necrotic spots and severe mosaic) (Fig. 1). The high susceptibility of Diana-1GH-2-3 plants with subsequent death of host tissue may be responsible for low virus titres on the 12th day of inoculation. In comparison, plants of line 2N23-2GH-3-2 were severely affected but not killed even after 33 days of inoculation and, therefore, retained higher titres of the virus.

Effect of Inoculum Concentration on Symptom Development

Plants of Diana-1GH-2-3 were infected when inoculated with each of the three dilutions of inoculum tested, although symptoms appeared in plants inoculated only at dilutions of 1:10 and 1:100 (Table 11). Plants of inbred line 2N23-2GH-3-2 were infected only at the 1:10 dilution but no symptoms developed even at the highest inoculum level. Inbred line 2N138-1GH-3-5 was immune to infection at all viral concentrations. To assess the virus titres in plant inoculated at each inoculum dilution, tissue from infected plants was assayed on 30 plants of Diana at a dilution of 1:100 (w/v) of leaf tissue to distilled water. When the inbred lines were inoculated with virus at a dilution of 1:10, V.I. in Diana-1GH-2-3

Table 11. Effect of inoculum concentration on symptom development induced by 'mosaic' strain of bean yellow mosaic virus at a diurnal temperature regime of 27 - 23° C

| Virus concentration | 2N138-1GH-3-5 | | | | 2N23-2GH-3-2 | | | | Diana-1GH-2-3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reaction* | D.S.† | D.V.§ | V.I.¶(%) | Reaction* | D.S.† | D.V.§ | V.I.¶(%) | Reaction* | D.S.† | D.V.§ | V.I.¶(%) |
| Virus dilution | | | | | | | | | | | | |
| 1:10 | I | - | - | 0.0 | L | - | 8 | 16.7 | S | 10 | 6 | 76.7 |
| 1:100 | I | - | - | 0.0 | I | - | - | 0.0 | S | 12 | 6 | 65.5 |
| 1:1000 | I | - | - | 0.0 | I | - | - | 0.0 | L | - | 8 | 13.3 |
| Number of leaves inoculated/plant | | | | | | | | | | | | |
| 1 | I | - | - | 0.0 | I | - | - | 0.0 | S | 12 | 8 | 36.7 |
| 2 | I | - | - | 0.0 | I | - | - | 0.0 | S | 12 | 6 | 53.3 |
| 4 | I | - | - | 0.0 | L | - | 8 | 16.7 | S | 12 | 6 | 76.7 |
| Number of aphids per plant | | | | | | | | | | | | |
| 2 | I | - | - | 0.0 | I | - | - | 0.0 | I** | - | - | 0.0 |
| 4 | S | 10 | 6 | 100.0 | L | - | 8 | 17.2 | S | 10 | 8 | 83.3 |
| 8 | S | 10 | 6 | 96.7 | L | - | 8 | 24.1 | S | 10 | 8 | 100.0 |

*I = Immune; L = Latent; S = Susceptible.

†D.S. = Days taken to express symptoms; - = symptoms not expressed up to 33 days after inoculation.

§D.V. = Days taken for virus to become detectable; - = virus not recovered up to 33 days after inoculation.

¶V.I. = Virus infectivity in leaf extracts diluted 1:100 (w/v) with distilled water, assayed 12 days after inoculation.

**One of the six plants tested had latent virus infection with 76.7% V.I. on the 12th day and expressed symptoms 14 days after inoculation. Other Diana plants remained virus- and symptom-free.

reached 76.7% as compared to only 16.7% in plants of the symptomless 2N23-2GH-3-2 (Table 11). Reduced virus multiplication and/or movement appears again to be the basis of resistance in symptomless line 2N23-2GH-3-2. The virus reached about the same infectivity level in line Diana-1GH-2-3 (13.3%), inoculated with a 1:1000 dilution of virus as it did in line 2N23-2GH-3-2 (16.7%) inoculated with a 1:10 dilution, thereby indicating that the rate of virus multiplication was approximately 100 times faster in plants of line Diana-1GH-2-3.

Different concentrations of virus were also introduced into the inbred lines by varying the number of leaves inoculated per plant. The results revealed that plants of Diana-1GH-2-3 developed symptoms in 12 days irrespective of the number of leaves per plant inoculated (Table 11). Line 2N23-2GH-3-2 was not infected unless four leaves per plant were inoculated in which case virus was recovered in young tissue within 8 days even though symptoms were absent. V.I. in Diana-1GH-2-3 plants remained much higher than in plants of the resistant inbred line 2N23-2GH-3-2, even when only one leaf of Diana-1GH-2-3 plant was inoculated (Table 11).

When different numbers of viruliferous aphids were used to vary the concentration of virus introduced into plants of each inbred line, two aphids were not able to infect most of the plants of even susceptible line Diana-1GH-2-3 (Table 11). However, one of the six Diana inbred plants tested had latent virus infection with a V.I. value of 76.7% on the 12th day and developed symptoms 14 days after inoculation. Four and eight aphids on the other hand, infected all or most of the plants including plants of line 2N138-1GH-3-5 which is immune to mechanical inoculation. Symptoms appeared in 10 days in lines Diana-1GH-2-3 and 2N138-1GH-3-5, whereas line 2N23-2GH-3-2 remained symptomless even 33

days after inoculation. Virus assay tests showed that virus could be detected in line 2N138-1GH-3-5 earlier (6 days after inoculation) than in the other inbred lines (8 days after inoculation). V.I. in symptomless line 2N23-2GH-3-2 (25%) was considerably less than V.I. in the other two lines (80-100%) when four or eight aphids per plant were used to inoculate virus and plants were assayed on the 12th day after inoculation (Table 11). An unexpected result was that line 2N138-1GH-3-5 although immune to mechanical inoculation not only became infected but appeared more susceptible than even Diana-1GH-2-3 when four or eight aphids were placed on the plants for 24 hours. In 1978 field trials, accession 2N138 from which inbred line 2N138-1GH-3-5 was derived, was resistant in plots exposed to viruliferous aphids (Thesis Section 3.1). BYMV was readily transmitted mechanically or with aphids in the majority of faba bean lines evaluated in the field in 1978. Further experiments were, therefore, conducted in attempts to explain why plants of inbred line 2N138-1GH-3-5 were immune when mechanically inoculated and susceptible when inoculated with viruliferous aphids in the greenhouse.

To determine whether epidermis may be a mechanical barrier to infection, epidermal strips were removed with forceps from the upper leaf surface of immune plants and the leaves were inoculated mechanically as described previously. The results show that three of the nine plants inoculated with the 'mosaic' strain when the epidermal strips were removed became infected as opposed to none in the intact plants (Table 12). However, plants of the immune line were not infected with the 'necrotic' strain of BYMV irrespective of whether epidermal strips were removed or not. It would thus appear that for the 'mosaic' strain at least, the resistance factor(s) is(are) located in the epidermal cells.

Table 12. Effect of removing leaf epidermis on the infection of faba bean inbred lines by mechanical inoculation with bean yellow mosaic virus

| Inbred line | Plants Infected/Plants Inoculated | | | |
|---|---|---|---|---|
| | 'Mosaic' Strain | | 'Necrotic' Strain | |
| | With epidermis | Without epidermis | With epidermis | Without epidermis |
| 2N138-1GH-3-5 | 0/9 | 3/9 | 0/5 | 0/4 |
| DIANA-1GH-2-3 | 12/12 | NT* | 6/6 | NT* |

*NT = Not tested.

The 24 hour aphid feeding period used for inoculation previously could be considered excessive since aphids were not confined to the leaves and might have moved to the young shoot tips whereas for mechanical inoculations, only expanded leaves are used. Therefore, a trial was made to assess the effects of shorter feeding periods. A 10 minute inoculation feeding period with two, four or eight aphids per plant resulted in no more than one infected plant even in the susceptible inbred line (Table 13). A feeding period of 4 hours with eight aphids per plant, however, infected all Diana-1GH-2-3 and 2N138-1GH-3-5 plants. A distinct mosaic appeared in plants of both lines. No clear differential response was seen in the two inbred lines irrespective of the number of aphids feeding or the length of the feeding period. One to two plants of the immune inbred line were again infected and developed symptoms when four or eight viruliferous aphids were placed on each of four to six plants tested at a lower temperature regime (21-17$^{\circ}$ C) using a 10 minute feeding period only.

In an effort to explain the discrepancy between the performance of the immune inbred line (2N138) in the field and in the greenhouse, field and greenhouse selections of the immune (2N138) and symptomless (2N23) accessions were evaluated again in the field in 1981 in which plants were exposed to viruliferous aphids. The accessions were planted in single 1-m-rows along with several rows of the susceptible cultivar 'Diana'. Pea aphids viruliferous with the 'mosaic' strain of BYMV were released on spreader rows of cultivar 'Diana' as described previously (Thesis Section 3.1). Plants were observed for the presence of pea aphids 7 and 14 days after aphids were released. Results summarized in Table 14 indicate that all the selections from 2N138 and 2N23 had a high percentage of the plants (68-100%) infested by the pea aphids, whereas not more than

Table 13. Effect of number of aphids and length of feeding period on infection by the 'mosaic' strain of bean yellow mosaic virus at 27 - 23° C diurnal temperature regime

| Inbred line | Aphid per plant | Plants Infected/Plants Tested | | | |
|---|---|---|---|---|---|
| | | Feeding Period | | | |
| | | 10 min | 1 h | 4 h | 24 h |
| 2N138-1GH-3-5 | 2 | 1/5 | 0/4 | 1/4 | 0/4 |
| | 4 | 0/4 | 2/4 | 3/4 | 3/4 |
| | 8 | 1/5 | 3/4 | 4/4 | 5/6 |
| DIANA-1GH-2-3 | 2 | 1/4 | 1/4 | 2/4 | 0/4 |
| | 4 | 0/4 | 2/4 | 4/6 | 4/4 |
| | 8 | 0/4 | 4/7 | 4/4 | 5/5 |

Table 14. Infestation by pea aphids and reaction of field and greenhouse faba bean selections exposed to aphids viruliferous with the 'mosaic' strain of bean yellow mosaic virus in the field (1981)

| Selection | Plants observed | Plants with pea aphids (%)¶ | Symptoms present/ virus recovered** |
|---|---|---|---|
| Field selections | | | |
| 2N138-1-1-1-1* | 25 | 17 ( 68.0) | -/- |
| 2N138-1-2-1-1* | 28 | 21 ( 75.0) | -/- |
| 2N23-3-2-1-1§ | 12 | 12 (100.0) | ±/+ |
| Greenhouse inbred selections | | | |
| 2N138-1GH-3-5* | 22 | 19 ( 86.4) | -/- |
| 2N23-2GH-3-2§ | 14 | 13 ( 92.9) | ±/+ |
| 2N23-2GH-3-2§ | 15 | 13 ( 86.7) | ±/+ |
| Commercial cultivar | | | |
| Diana-7 | 14 | 8 ( 57.1) | +/NA§§ |
| Diana-8 | 13 | 9 ( 69.2) | +/NA§§ |

*Selections previously identified as immune (free of symptoms and virus).

§Selections previously identified as symptomless with latent infections of BYMV.

**Majority of the symptomless plants assayed were infested by aphids; - = symptoms not expressed or virus not recovered; + = symptoms present or virus recovered; ± = some plants with mild symptoms, others without. Generally 2-6 plants per selection were assayed.

§§NA = Not assayed for virus.

¶Infestation recorded 7 and 14 days after aphids were released on the spreader rows.

70% of the Diana plants were infested. Plants from field and greenhouse selections from 2N138 remained free of symptoms of BYMV and were also free of virus when assayed at the late pod filling stage. All plants of the cultivar Diana developed severe symptoms of BYMV. Late in the season, a few plants of 2N23 and Diana developed mild chlorosis and some reduction in size of young leaves. This was identified as late infection by aster yellows which was very prevalent in 1981. These results are in agreement with results obtained in previous field testing (Thesis Section 3.1). However, it appeared that the resistance mechanism of accession 2N138 is fully expressed under field conditions and not in the greenhouse.

# DISCUSSION

The results of the temperature studies showed that temperature had little effect on the time required for symptom development and for virus to become detectable in plants of both resistant and susceptible inbred lines. These findings are consistent with those of Schroeder et al. (1960) and Schroeder et al. (1966) who found that symptom expression was influenced by temperature in heterozygous plants only.

Using different concentrations of virus inoculum to inoculate resistant and susceptible genotypes demonstrated that final host reactions and virus concentrations in the host were affected by the amount of virus introduced. When inoculum concentration is low, a susceptible host may escape infection and appear resistant. Virus titre rather than days necessary for virus to be first detectable in the inoculated test plants appeared to be correlated with final host reactions. When four viruliferous pea aphids per plant were used, virus infectivity in plants of inbred line Diana-1GH-2-3 was found to be 83.3% as compared to only 17.2% in plants of inbred line 2N23-2GH-3-2 on the 12th day of inoculation even when virus could be initially detected 8 days after inoculation in both inbred lines (Table 11). Reduced virus multiplication appeared to be the basis of resistance in line 2N23-2GH-3-2.

The susceptibility of inbred line 2N138-1GH-3-5 that is immune to mechanical inoculation when it was inoculated by viruliferous aphids in the greenhouse was unexpected and could not be fully explained. The susceptibility of the immune line, 2N138-1GH-3-5, to the 'mosaic' strain of BYMV when epidermal strips were removed from the upper leaf surfaces

before mechanical inoculation would seem to indicate that the resistance lies in the epidermal cells. On the other hand, field and greenhouse selections from 2N138 were not infected when exposed in the field to viruliferous pea aphids. Perhaps the epidermal cells have thicker walls in field-grown plants and prevent the aphids from probing deeper than the epidermis and reaching the mesophyll cells. Further studies are required to explain the response of the immune line. The results of the present studies indicate the necessity of evaluating faba bean lines for resistance to BYMV in the field as well as in the greenhouse, of using high inoculum concentrations of the virus and of inoculating plants mechanically as well as by viruliferous aphids.

## GENERAL DISCUSSION

The results of the first study demonstrate that faba bean selections with resistance (symptomless or mild mosaic) to the 'mosaic' strain of BYMV can be effectively identified through testing and reselection in the field over several years. Outcrossing must have occurred at low levels only, otherwise progress would not have been made.

Homogeneity for resistance to both 'mosaic' and 'necrotic' strain was achieved in six selections within three cycles of testing and selection in the greenhouse. However, as was shown in the last study, the immune inbred line became susceptible to virus transmission by aphids in the greenhouse, whereas it remained immune when retested with the aphids in the field. This indicates the need to test selections in the field as well as in the greenhouse.

This is the first study of the genetics of resistance in V. faba to BYMV. The resistance in line 2N23-2GH-3-2 to the 'mosaic' strain and immunity in line 2N138-1GH-3-5 to both virus strains were each conditioned by two recessive genes. Although the differential response of the inbred lines to the two virus strains suggests that the genes for resistance in each line are different, further genetic studies are required to confirm the genotypes of the immune and highly resistant inbred lines. Limitations are encountered when studying genetics of host-virus systems that are not common to other host-pathogen systems. The same plants cannot be tested to two or more strains as in rust or other pathogens because of the systemic nature of virus infections and the cross-protection phenomenon.

Selecting and inbreeding susceptible genotypes is also difficult because severe infections often result in premature death or minimum seed production. As demonstrated by the genetic studies, at least two recessive genes appeared to be involved in control of the latent reaction and immunity to BYMV. To determine the genotypes of inbred lines that are symptomless or immune several hundred plants of $F_1$ and $F_2$ progenies from a cross between the two lines need to be studied. A virus assay that is capable of detecting low virus titres is required to differentiate the segregating progenies into immune and symptomless plants.

If resistance to BYMV was controlled by two recessive genes, the selection made for resistance should ordinarily result in a uniformly resistant selection. However, heterozygosity was shown to delay symptoms by 7-14 days and it is possible that occasionally selections for resistance were made from accessions that were symptomless but heterozygous or developed symptoms late in the season. This may explain why the number of symptomless plants increased gradually during testing and reselection in the field even in selections where symptomless plants were in very low numbers or not present at all initially.

When infected with the 'mosaic' strain of BYMV, symptomless plants of line 2N23-2GH-3-2 always had lower virus concentrations than plants of Diana-1GH-2-3 even though virus was detected at about the same time in both lines. This would indicate that resistance was due to a reduced rate of virus multiplication rather than to reduced movement within the host tissue.

When a low inoculum concentration was used, plants of Diana-1GH-2-3, although infected, did not develop symptoms until 33 days after inoculation and had very low virus titre when assayed on the 12th day of

inoculation. This suggests that a high inoculum concentration is required to cause symptoms even in susceptible plants, and that symptom development was related to the virus titre. In contrast, studies by Kuhn et al. (1981) revealed that the failure of cowpea plants infected with cowpea chlorotic mottle virus to develop symptoms was due to the lack of movement of the virus and that virus accumulation was not related to symptom development. Although resistance to BYMV has been found in several legume species including peas (Yen and Fry, 1956; Johnson and Hagedorn, 1958; Schroeder and Provvidenti, 1962, 1971; Barton et al., 1964), Phaseolus beans (Baggett, 1956; Baggett and Frazier, 1957; Provvidenti and Schroeder, 1973), cowpeas (Reeder et al., 1972) and soybeans (Provvidenti, 1975), the relationship between virus multiplication and/or movement in the host and resistance was not studied.

The susceptibility of line 2N138-1GH-3-5 inoculated by pea aphids in the greenhouse and which was resistant when retested in the field is difficult to explain. The fact that plants were susceptible when inoculated mechanically after removal of epidermal strips from the upper leaf surfaces would seem to indicate that resistance lies in the epidermis and that aphids penetrate through the epidermis into the mesophyll. On the other hand, perhaps resistance is due to a lack or non-function of infectible sites on the surface of epidermal cells. In this case, the ability of aphids to penetrate past the epidermis cell wall and into the cytoplasm would enable them to circumvent the resistance. More detailed studies in the field and the greenhouse of the probing and feeding habits of the pea aphid as well as on the duration of the feeding period might help to explain the problem.

This phenomenon has not been reported in other legumes that were

reported resistant to BYMV primarily because screening and selection for resistance in all cases used mechanical inoculation of the virus. In the studies cited previously, viruliferous aphids were not used to inoculate germplasm with BYMV except for tests reported by Provvidenti (1975) in which soybean accessions were resistant when exposed to natural BYMV infections in the field.

Further research is required to elucidate why aphids were not able to transmit the virus to the immune faba bean line in the field. It would also be of interest to compare the performance of other field selections of 2N138 in the greenhouse to both the 'mosaic' and 'necrotic' strains to determine the universality of this response in the immune line.

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# APPENDIX

Appendix 1. Tests to determine optimum pressure and carborundum concentrations for air-brush inoculations of bean yellow mosaic virus at 27° C

| Virus strain | Pressure in kPa (psi) | Plants Infected/Plants Inoculated* Carborundum Concentration (%) 0 | 1 | 2 |
|---|---|---|---|---|
| 'mosaic' | 138 (20) | 0/30 | 1/30 | 7/30 |
| | 207 (30) | 0/30 | 4/30 | 26/30 |
| | 276 (40) | 2/30 | 11/30 | 30/30 |
| | 330 (50) | 6/30 | 19/30 | 30/30 |
| | Control† | 13/30 | 23/30 | 27/30 |
| 'necrotic' | 138 (20) | 0/30 | 0/30 | 8/30 |
| | 207 (30) | 0/30 | 5/30 | 28/30 |
| | 276 (40) | 2/30 | 13/30 | 30/30 |
| | 330 (50) | 5/30 | 17/30 | 30/30 |
| | Control† | 11/30 | 24/30 | 28/30 |

*Leaf extracts from BYMV-infected plants in 0.1 M phosphate buffer (pH 7.4) (1:10, w/v) were used as inoculum.

†Plants inoculated with hand using a pipecleaner or a cotton swab.

Appendix 2. Percentage of faba bean plants resistant to the 'mosaic' strain of BYMV in 1978 field trials inoculated mechanically or with aphids

| Accessions ††† | Percent Plants | | | | Accession | Percent Plants | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mech Test | | Aphid Test | | | Mech Test | | Aphid Test | |
| | HR* | MR† | HR* | MR† | | HR* | MR† | HR* | MR† |
| 2N23-1 | 4.5 | 20.5 | 0.0 | 13.3 | UMFB12-1(OR77) | 0.0 | 13.3 | 0.0 | 33.3 |
| 2N40-1 | 0.0 | 8.6 | 0.0 | 6.7 | UMFB20-1(OR77) | 0.0 | 13.3 | 0.0 | 0.0 |
| 2N43-1 | 0.0 | 2.2 | 0.0 | 0.0 | 2N43-3(OR77) | 0.0 | 0.0 | - | - |
| 2N85-1 | 5.4 | 24.3 | 0.0 | 26.7 | 2N62-1(OR77) | 0.0 | 42.7 | 8.3 | 58.3 |
| 2N101-1 | 2.5 | 20.0 | 0.0 | 13.3 | UMFB13-1(OR G.H.) | 0.0 | 13.3 | 0.0 | 33.3 |
| 2N23-2 | 0.0 | 24.4 | 0.0 | 33.3 | L5-22-2(OR G.H.) | 0.0 | 13.3 | 0.0 | 53.3 |
| 2N40-2 | 3.3 | 16.7 | 0.0 | 0.0 | L5-42-1(OR G.H.) | 0.0 | 0.0 | - | - |
| 2N43-2 | 4.9 | 7.3 | 0.0 | 6.7 | L5-13-2(1R77) | 0.0 | 40.0 | 0.0 | 13.3 |
| 2N85-2 | 10.8 | 32.4 | 0.0 | 33.5 | L5-18-2(1R77) | 0.0 | 33.3 | 0.0 | 0.0 |
| 2N101-2 | 12.5 | 20.5 | 6.7 | 33.5 | Blue Rock-2(1R77) | 0.0 | 6.7 | 0.0 | 20.0 |
| 2N138-1 | 6.9 | 6.9 | 18.2 | 27.3 | 2N4-2(1R77) | 0.0 | 33.3 | 0.0 | 0.0 |
| 2N415-1 | 0.0 | 17.7 | 0.0 | 26.7 | 2N40-4(1R77) | 6.3 | 6.3 | 0.0 | 0.0 |
| UMFB15-1 | 0.0 | 2.8 | 0.0 | 58.3 | 2N40-5(1R77) | 0.0 | 0.0 | 0.0 | 47.7 |
| DIANA | 0.0 | 0.0 | 0.0 | 0.0 | 2N23-4(1R77) | 0.0 | 40.0 | 6.7 | 53.3 |
| 2N1-1 | 0.0 | 2.1 | 0.0 | 0.0 | 2N44-2(1R77) | 0.0 | 6.7 | 0.0 | 0.0 |
| 2N21-1 | 2.3 | 14.0 | 0.0 | 33.3 | 2N101-4(1R77) | 0.0 | 0.0 | 0.0 | 0.0 |
| 2N23-3(OR77) | 0.0 | 23.7 | 6.7 | 26.7 | 2N124-2(1R77) | 0.0 | 7.7 | 0.0 | 0.0 |
| 2N101-3(OR77) | 17.1 | 22.0 | 13.3 | 33.5 | 2N295-2(1R77) | 0.0 | 57.2 | 0.0 | 66.7 |
| 2N40-3(OR77) | 0.0 | 4.2 | 20.0 | 40.0 | 2N425-2(1R77) | 12.5 | 75.0 | - | - |
| 2N100-1 | 2.7 | 0.0 | 0.0 | 47.7 | 2N429-1(1R77) | 0.0 | 50.0 | - | - |
| L5-22-1(OR77) | 6.7 | 20.0 | 0.0 | 27.3 | 2N265-1(1R77) | 0.0 | 9.1 | - | - |
| L5-13-1(OR77) | 6.7 | 53.3 | 6.7 | 33.3 | OPM-77-14 Low-1 | 0.0 | 7.1 | - | - |
| L5-18-1(OR77) | 12.5 | 18.8 | 26.7 | 40.0 | OPM-77-1 Low-1 | 0.0 | 13.3 | 20.0 | 53.3 |
| Blue Rock-1(OR77) | 6.7 | 6.7 | 6.7 | 40.0 | 2N78-1(MILD) | 10.0 | 10.0 | 0.0 | 0.0 |
| 2N4-1(OR77) | 13.3 | 66.7 | 0.0 | 26.7 | UMFB18-1(MILD) | 0.0 | 0.0 | 0.0 | 20.0 |
| 2N44-1(OR77) | 33.3 | 66.7 | 0.0 | 33.3 | UMFB19-1 | 6.7 | 14.3 | - | - |
| 2N421-1(OR77) | 26.7 | 47.7 | 20.0 | 66.7 | DIANA | 0.0 | 0.0 | - | - |
| 2N124-1(OR77) | 7.1 | 57.1 | 0.0 | 13.3 | 2N1-2 | 0.0 | 31.3 | - | - |
| 2N297-1(OR77) | 21.4 | 42.9 | 0.0 | 6.7 | 2N21-2 | 0.0 | 6.7 | - | - |
| 2N295-1(OR77) | 13.3 | 80.0 | 26.7 | 60.0 | UMFB13-2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2N263a-1(OR77) | 0.0 | 6.7 | -§ | - | UMFB15-2 | 0.0 | 0.0 | 6.7 | 80.0 |
| 2N263b-1(OR77) | 0.0 | 0.0 | - | - | UMFB19-2 | 0.0 | 6.7 | 0.0 | 0.0 |
| 2N239-1(OR77) | 0.0 | 20.0 | 0.0 | 40.0 | UMFB20-2 | 0.0 | 6.7 | - | - |
| 2N5-1(OR77) | 0.0 | 0.0 | 6.7 | 33.3 | 2N425-3(OR77) | 20.0 | 33.3 | 0.0 | 0.0 |
| | | | | | 2N236-1 | - | - | 0.0 | 0.0 |

*Highly resistant with a symptom rating of '0' in any of three types, mosaic, stunting and necrosis.

†Moderately resistant with a maximum rating of '1' in any of the three symptom types.

§- = Accession not tested.

†††Results of the first 20 accessions under mechanical inoculation test are based on three replications.

Appendix 3. Percentage of faba bean plants resistant to the 'mosaic' strain in mechanically-inoculated 1979 field trials

| Accessions †† | Percent Plants HR * | MR † | Accessions †† | Percent Plants HR * | MR † |
|---|---|---|---|---|---|
| DIANA-1 | 0.0 | 4.2 | 2N85-1-1(OR78) | 35.7 | 28.6 |
| DIANA-2 | 10.3 | 10.3 | 2N101-1-1(1R78) | 44.7 | 33.3 |
| 2N23-1-1(1R78) | 2.9 | 22.9 | 2N425-2-1(OR78) | 57.1 | 28.6 |
| UMFB15-1-1(1R78) | 3.0 | 39.4 | UMFB12-1-1(1R78) | 5.3 | 31.6 |
| 2N295-2-1(1R78) | 34.6 | 42.3 | 2N78-1-1(OR78) | 25.0 | 50.0 |
| 2N23-3-1(1R78) | 3.6 | 14.3 | 2N101-3-2(1R78) | 0.0 | 8.3 |
| 2N23-2-1(1R78) | 8.3 | 23.3 | 2N23-3-4(1R78) | 0.0 | 15.8 |
| 2N23-3-2(OR78) | 2.5 | 37.5 | 2N101-3-3(1R78) | 15.4 | 15.4 |
| UMFB13-1-1(1R78) | 4.0 | 0.0 | 2N101-1-2(1R78) | 9.1 | 54.6 |
| 2N43-2-1(1R78) | 20.0 | 52.0 | 2N425-3-1(1R78) | 8.3 | 25.0 |
| DIANA-3(1R78) | 14.3 | 21.4 | 2N101-2-1(OR78) | 16.7 | 33.3 |
| 2N23-1-2(1R78) | 0.0 | 10.0 | 2N138-1-2(OR78) | 50.0 | 50.0 |
| GH7871-1(1R78) | 2.4 | 0.0 | L5-22-2-1(1R78) | 27.3 | 9.1 |
| 2N23-3-3(OR78) | 0.0 | 94.7 | 2N101-2-2(1R78) | 18.2 | 18.2 |
| L5-22-1-1(OR78) | 10.5 | 36.8 | 2N101-3-4(OR78) | 0.0 | 50.0 |
| L5-22-1-2(OR78AP) | 37.9 | 24.1 | c/104/308/73 Giza-1 | 50.0 | 12.5 |
| 2N138-1-1(OR78) | 77.8 | 5.6 | 2N101-2-3(1R78) | 16.7 | 46.2 |
| 2N23-1-3(OR78) | 0.0 | 50.0 | 2N40-1-1(1R78) | 12.7 | 50.0 |
| DIANA-4 | 3.2 | 0.0 | 2N425-3-2(1R78) | 25.0 | 50.0 |
| 2N85-2-1(1R78) | 0.0 | 7.1 | L5-13-2-1(1R78) | 35.3 | 0.0 |
| 2N101-3-1(1R78) | 33.3 | 16.7 | 2N101-3-5(1R78) | 46.2 | 23.1 |
| 2N295-1-1(OR78) | 33.3 | 30.0 | 2N1-2-1(OR78) | 23.1 | 23.1 |
| DIANA-5 | 0.0 | 7.1 | 2N78-1-2(1R78AP) | 0.0 | 12.5 |
| GH7844(1R78) | 0.0 | 23.1 | 2N23-2-2(1R78) | 0.0 | 0.0 |
| DIANA | 20.0 | 6.7 | NA52A Giza-1 | Root Rot | |
| | | | 2N78-1-3(1R78) | 0.0 | 0.0 |

*Highly resistant with a '0' rating in all symptom types.

†Moderately resistant with a maximum rating of '1' in any symptom type.

††In parenthesis are reactions of previous year. 0 = no disease; 1 = mild symptoms.

Appendix 4. Percentage of faba bean plants resistant to the 'mosaic' strain in mechanically-inoculated 1980 field trials

| Accessions | Percent Plants | |
|---|---|---|
| | HR* | MR† |
| 2N23-1-1-1(2R79) | 0.0 | 0.0 |
| 2N295-2-1-1(OR79) | 25.0 | 68.8 |
| 2N23-3-1-1(1R79) | 37.5 | 31.3 |
| 2N23-3-2-1(OR79) | 66.7 | 33.3 |
| UMFB13-1-1-1(3S79) | 0.0 | 0.0 |
| UMFB13-1-1-2(1R79) | 15.4 | 0.0 |
| 2N43-2-1-1(OR79) | 55.6 | 27.8 |
| L5-22-1-1-1(OR79) | 30.4 | 52.2 |
| L5-22-1-2-1(1R79) | 25.0 | 0.0 |
| 2N138-1-1-1(OR79) | 94.1 | 5.9 |
| 2N23-1-3-1(1R79) | 22.2 | 33.3 |
| DIANA(3S79) | 0.0 | 0.0 |
| 2N85-2-1-1(1R79) | 0.0 | 14.3 |
| 2N295-1-1-1(1R79) | 0.0 | 0.0 |
| DIANA(3S79) | 0.0 | 0.0 |
| DIANA(3S79) | 0.0 | 0.0 |
| 2N101-1-1-1(OR79) | 85.7 | 14.3 |
| 2N425-2-1-1(1R79) | 16.7 | 33.3 |
| 2N138-1-2-1(1R79) | 8.3 | 0.0 |
| C104/308/73 Giza-1(OR79) | by root rot | |
| 2N425-3-2-1(1R79) | 25.0 | 0.0 |
| 2N101-3-5-1(1R79) | 0.0 | 0.0 |
| 2N1-2-1-1(1R79) | 7.7 | 15.4 |
| NA52A Giza-1-1(OR79) | Masked by root rot | |
| NA52A Giza-1-2(1R79) | Masked by root rot | |

*Highly resistant with a rating of '0' in all symptom types.

†Moderately resistant with a maximum of '1' rating in any type.

Appendix 5. Percentage of faba bean plants resistant to the 'mosaic' strain in 1981 field trials inoculated with aphids

| Accessions†† | Percent Plants HR* | MR† | Accessions†† | Percent Plants HR* | MR† |
|---|---|---|---|---|---|
| 2N85-2-1-1-1(OR80) | 61.5 | 23.1 | OPM77-1 Low-1GH-1(OR78) | 71.4 | 14.3 |
| 2N23-3-2-1-1(OR80) | 73.3 | 20.0 | 2N78-1GH-i(1R78) | 25.0 | 43.8 |
| 2N43-2-1-1-1(OR80) | 0.0 | 36.4 | UMFB19-1GH-1(1R78) | 10.0 | 26.7 |
| 2N85-2GH-1-1(OR GH79) | 21.4 | 21.4 | 2N12-1 | 58.3 | 25.0 |
| 2N101-2GH-1(OR GH79) | 91.3 | 8.3 | 2N15-1 | 40.0 | 20.0 |
| 2N138-1GH-1(1R78) | 0.0 | 66.7 | 2N18-1 | 0.0 | 37.5 |
| 2N138-1GH-3-5(OR GH79) | 100.0 | 0.0 | 2N61-1 | 46.2 | 46.2 |
| 2N425-3-2-1-1(OR80) | 61.5 | 30.8 | 2N63-1 | 41.7 | 25.0 |
| UMFB15-1GH-1(OR78) | 33.3 | 53.3 | 2N138-1-1-1-1(OR80) | 100.0 | 0.0 |
| 2N101-3GH-1(OR78) | 78.6 | 14.3 | 2N138-1-1-1-2(OR80) | 100.0 | 0.0 |
| 2N101-1-1-1-2(OR80) | 76.9 | 15.4 | 2N138-1-2-1-1(OR80) | 75.0 | 25.0 |
| 2N40-3GH-1(OR78) | 35.7 | 28.6 | 2N138-1-2-1-2(1R80) | 60.0 | 20.0 |
| 2N100-1GH-1(OR78) | 69.2 | 30.8 | 2N138-1-1(OR78) | 35.7 | 35.7 |
| L5-22-1-1-1-1(OR80) | 35.3 | 47.1 | UMFB13-1GH-1 | 5.9 | 5.9 |
| L5-13-1GH-1(1R78) | 33.5 | 50.0 | DIANA-4 | 10.0 | 10.0 |
| L5-18-1GH-1(OR78) | 46.2 | 46.2 | UMFB12-1GH-1(OR78) | 21.4 | 50.0 |
| Blue Rock-1GH-1(OR78) | 27.3 | 63.6 | 2N62-1GH-1(OR78) | 33.3 | 26.7 |
| 2N4-1GH-1(OR78) | 50.0 | 50.0 | L5-13-1GH-1(OR78) | 30.8 | 30.8 |
| 2N44-1GH-1(OR78) | 53.8 | 23.1 | L5-18-1GH-1(OR78) | 6.7 | 53.3 |
| 2N421-2(OR77) | Root Rot | | 2N40-5GH-1(1R78) | 25.0 | 25.0 |
| 2N124-1GH-1(OR78) | 58.3 | 33.3 | 2N295-2GH-1-5(OR GH79) | 91.7 | 8.3 |
| 2N297-1GH-1(OR78) | 36.4 | 45.5 | 2N425-2GH-1-1(OR GH79) | Root Rot | |
| 2N295-2-1-1-1(OR80) | 85.7 | 14.3 | 2N429-1GH-1(OR GH78) | Root Rot | |

*Highly resistant plants with a rating of '0' in all symptom types.

†Moderately resistant plants with a maximum rating of '1' in any symptom type.

††All accessions with 'GH' were tested and inbred in the greenhouse.

Appendix 6. Plant species tested for local lesion production by 'mosaic' strain of BYMV at 27° C

| Host species | Virus dilution* | Leaves with local lesions/ leaves inoculated | Local lesions per leaf | Range of local lesions between leaves |
|---|---|---|---|---|
| Chenopodium amarantiolor | 1:10 | 10/10 | 44.3 | 9-63 |
| | 1:100 | 15/20 | 13.5 | 0-22 |
| | 1:10 | 6/6 | 10.3 | 6-13 |
| | 1:50 | 6/6 | 5.6 | 3-8 |
| | 1:100 | 6/6 | 9.7 | 4-13 |
| | 1:200 | 6/6 | 3.0 | 2-5 |
| | 1:400 | 3/6 | 1.5 | 0-3 |
| C. quinoa | 1:10 | 0/20 | 0.0 | 0-0 |
| | 1:100 | 0/20 | 0.0 | 0-0 |
| Phaseolus vulgaris § | | | | |
| Bountiful | 1:10 | 4/6 (6/6) † | 7.0 ( 50.0)† | 0-26 (20- 100)† |
| | 1:100 | 4/6 (5/6) | 31.5 ( 200) | 0-68 ( 0- 225) |
| Kentucky Wonder †† | 1:10 | 0/6 (3/6) | 0.0 (5.8) | 0-0 ( 0-13) |
| | 1:100 | 0/6 (0/0) | 0.0 (0.0) | 0-0 (0-0) |
| Dark Red Kidney | 1:10 | 4/6 (6/6) | 9.3 (63.0) | 0-24 (12-170) |
| | 1:100 | 4/6 (5/6) | 61.2 ( 200) | 0-210(0- 400) |
| Prince | 1:10 | 0/20 | 0.0 | 0-0 |
| | 1:100 | 0/20 | 0.0 | 0-0 |
| P. aureus | 1:10 | 0/20 | 0.0 | 0-0 |
| | 1:100 | 0/20 | 0.0 | 0-0 |
| Cyamopsis tetragonoloba | 1:10 | 0/24 | 0.0 | 0-0 |
| | 1:100 | 0/24 | 0.0 | 0-0 |
| Cucumis sativum | 1:10 | 0/20 | 0.0 | 0-0 |
| | 1:100 | 0/20 | 0.0 | |

*Crude leaf extracts from 14-day-old infected faba bean plants were prepared and diluted in 0.1 M phosphate buffer of pH 7.4.

†Values in parenthesis were obtained after chlorophylls from leaves with L.L. were removed and leaves were stained with iodine sol.

§Only once lesions appeared and were stained. Lesions did not appear in any of the varieties when the tests were repeated later.

††Plants had severe veinal necrosis in young leaves.

Appendix 7. Attempted modifications to the standard enzyme-linked immunosorbent assay (ELISA) technique described by Clark and Adams (1977)

The following modifications and/or additional changes were attempted, singly or in various combinations, in the ELISA tests to assay 'mosaic' and 'necrotic' strains using antisera* to BYMV, very kindly supplied by Dr. R. Stace-Smith, Agriculture Canada, Vancouver.

A. Pretreatment of antisera with leaf extracts from faba bean to absorb host-specific antibodies.

( i) Antiserum was pretreated with a crude plant extract or with partially purified and freeze-dried host proteins and then used to extract γ-globulin.

(ii) Purified γ-globulin ($OD_{280}$=1.4) was pretreated with plant extracts or with partially purified and freeze-dried host proteins. The treated γ-globulin was repurified using a pre-equilibrated column of DE-22 cellulose and then concentrated to $OD_{280}$=1.4.

The absorbed γ-globulin was subsequently used in coating buffer or in making γ-globulin-enzyme conjugates.

B. The faba bean was replaced by two other systemic hosts, Kentucky Wonder beans (Phaseolus vulgaris) and Laxton peas (Pisum sativum) in attempts to eliminate the assumed interfering factors from faba beans.

(Continued)

*BYMV, used as antigen for antisera production, was propagated in Vicia faba and Nicotiana clevilandi.

Appendix 7. (Continued)

C. The γ-globulin-enzyme conjugate (A-E) was obtained after 1.0 ml of purified γ-globulin ($OD_{280}$=1.4) was mixed with 5 mg of alkaline phosphatase (E.C. 3.1.3.1, Sigma type VII) in the presence of glutaraldehyde (0.05% final concentration). A-E was then diluted to 1:10, 1:100, 1:200, 1:400, 1:1000, 1:3200 and 1:5000 in 0.1 M phosphate buffered saline (PBS) (pH 7.4) so as to find the highest dilution that yields positive results and could be used subsequently. Although a dilution of 1:200 was found to be adequate, none of the dilutions could differentiate virus-specific or non-specific (virus-free) reactions.

(Continued)

Appendix 7. (Continued)

D. Virus extractions and concentrations

Virus preparations and concentrations used in the ELISA tests were prepared as follows

| Preparation | Extraction media/ method used | Concentration |
|---|---|---|
| Crude-1* | 0.1 M phosphate buffer (pH 7.4) | $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ |
| Crude-2 | 0.01 M PBS (pH 7.4) mixed with 0.05% Tween-20 (PBS-T) | As in crude-1 |
| Crude-3 | PBS-T mixed with 2% Polyvinylpyrrolidone (PBS-TP) | As in crude-1 |
| Crude-4 | PBS-TP mixed with 0.2% ovalbumin (PBS-TPO) | As in crude-1 |
| Crude-5 | PBS-TPO mixed with chloroform (2:1) and then centrifuged at 2000 rpm for 10 min | As in crude-1 |
| Partially purified-1 | Frowd and Bernier (1977) | $10^{-1}$, $10^{-2}$, $10^{-3}$ |
| Partially purified-2 | Frowd and Bernier (1977) except 2-mercaptoethanol was replaced with 0.01 M ascorbic acid | $10^{-1}$, $10^{-2}$, $10^{-3}$ |

*Leaf tissue from virus-infected faba bean plants was triturated in the described medium (1:10, w/v), the extract was filtered through 2-4 layers of cheesecloth and then used to make further dilutions in 0.01 M PBS (pH 7.4). For control, virus-free faba bean plants were used and identical dilutions were made.

E. Four concentrations, 0.1 µg, 1 µg, 5 µg and 10 µg of purified absorbed and non-absorbed γ-globulin ($OD_{280}$=1.4) per ml of coating buffer (Clark and Adams, 1977) were used to obtain a concentration that could efficiently be used in the subsequent tests. Although all the

(Continued)

Appendix 7. (Continued)

concentrations gave positive results, none could again provide distinct values for virus-specific reactions.

F. Four reaction intervals, 1 h, 4 h, 8 h (all at room temperature, 21 - 22° C) and 24 h (at 4 - 6° C) were allowed after 300 µl of p-nitrophenyl phosphate substrate was added to the test wells. Reactions were stopped using 50 µl or 100 µl of 3M NaOH. The visual and photometric measurements at 405 nm differed between reaction intervals, 8 h providing the highest values, however, none gave distinctions between test and control reactions.

G. Other changes such as increased washings of the wells with PBS-T after each step, not using the peripheral wells were also not successful. In all tests, polystyrene microelisa plates (M129A) from the Dynatech Ltd. and Hitachi model 100-40 or CARL ZEISS (West Germany) spectrophotometer were used.