Evaluating *Pisum sativum* for Resistance to Pea Mosaic

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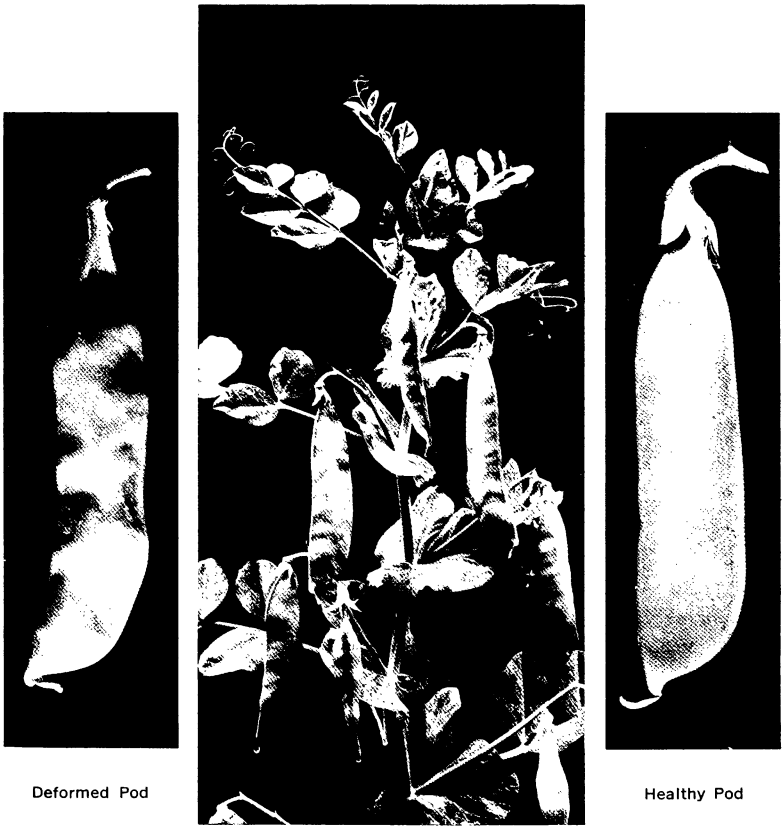

Deformed Pod

Diseased Plant

Healthy Pod

New York State Agricultural Experiment Station

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Cover . . .

Symptoms of pea mosaic, caused by strains of the bean yellow mosaic virus. Intensity of foliage mottle varies with variety and virus strain; leaf size may also be reduced. Pod distortion often develops (Insets—*left,* deformed pod, *right,* healthy pod).

Summary . . .

Material in this bulletin describes an inoculation and evaluation procedure that will ensure an accurate characterization of pea breeding progenies for reactions to pea mosaic (BV2=BYMV) conditioned by the *mo mo* genotype. Reactions of 248 varieties and breeding lines of pea to BV2 were analyzed. The results emphasized that little reliance can be placed on varietal names for resistance and that the resistant *mo mo* genotype must be considered as an independent character in a breeding program.

Evaluating Pisum sativum for Resistance to Pea Mosaic

By W. T. Schroeder and R. Provvidenti*

THE IMPORTANCE of pea mosaic, caused by the bean yellow mosaic virus (BYMV = BV2), has been emphasized by investigators from coast to coast and in some foreign countries, especially in connection with the susceptibility of some varieties of pea in the Perfection group.[3,6,7,8,11,14] Following the demonstration by Pierce[12] in 1935 that a Perfection variety was resistant to BV2, it was generally assumed that any variety derived from Perfection or with some form of Perfection in its name would likewise be resistant. The most plausible explanation of the lack of resistance in some Perfection lines was that when the resistant prototype was crossed with susceptible varieties, the incidence of pea mosaic in the breeding plots was insufficient to eliminate all of the susceptible individuals from a given progeny during the selecting process.[3,8,11] Thus, some BV2-susceptible plants were unwittingly selected and increased as new varieties of the Perfection type. This problem continually recurs in any pea breeding program involving mosaic susceptible and resistant parents. A need exists for an inoculation procedure to provide conclusive evidence for homozygous resistance to BV2 in pea.

Considerable information is available concerning factors that affect BV2 resistance in pea and which might provide a basis for an evaluation procedure. Yen and Fry[18] showed that resistance to a pea mosaic virus, infective to bean but otherwise similar to a pea mosaic virus (PMV) described by Chamberlain,[2] was conditioned by the *mo mo* genotype. Johnson and Hagedorn[10] later indicated that resistance to BV2 (pea isolate 1) was similarly inherited. Goodchild[5] demonstrated a serological relationship between BV2 and PMV, the latter virus probably being identical with the PV2 of Stubbs[17] and PV3 of Pierce.[12] Cross-protection tests have corroborated this relationship.[5,14] Additional evidence to support the identity of PV2 (=PMV, PV3) as a strain of BV2 was obtained when clonal lines of pea derived from segregating populations of susceptible x resistant crosses showed that resistance to the two viruses was conditioned by genotype *mo mo*.[1] Yen and Fry[18] and Johnson and Hagedorn[10] observed that heterozygotes (*Mo mo*) exhibited a delayed response to inoculation, but the demarcation between *Mo mo* and *Mo Mo* was not clear-cut enough to separate them.

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The delayed response of the heterozygotes was later demonstrated to be a function of air temperature rather than time.[15] Temperatures of 65°F. or lower suppressed symptoms in the heterozygotes, but 80°F. or higher induced symptoms in them as quickly as in the *Mo Mo* genotype. Moreover, symptom expression in the heterozygotes was reversible by temperature manipulation. That is, symptoms developed on foliage of *Mo mo* plants incubated at 80°F. or higher, but were absent on new growth that developed after the plants were removed to 65°F. or lower. Conversely, plants of the *Mo mo* genotype inoculated and incubated at 65°F. or lower remained symptomless until moved to the higher temperature, with one notable exception. *Mo mo* plants inoculated with the PV2 strain and incubated at 65°F. or lower were not infected.

Temperature has a further influence on the resistance of pea to some strains of BV2. Thermal isolates of BV2 capable of overcoming the resistance conditioned by the *mo mo* genotype were obtained by exposing pea plants infected with certain strains of BV2, notably pea isolate 1, to high temperatures (85°F. or higher) for prolonged periods and recovering the virus on *mo mo* plants.[13] PV2 was exceptional in that it never yielded a thermal isolate. These isolates are presently only of academic interest, but occasional strains of BV2 isolated from naturally infected pea or alsike clover plants that are capable of overcoming *mo mo* resistance may have arisen in this manner. Fortunately, thermal strains are not presently widespread in nature, probably because they are not efficiently transmitted by the pea aphid.[16]

This bulletin: (1) presents available information basic to an inoculation procedure developed to ensure an accurate evaluation of breeding progenies for BV2 resistance in pea conditioned by the *mo mo* genotype, (2) emphasizes that resistance must be treated as an independent character, not as a function of a variety or strain of pea and finally, (3) provides interested individuals with a listing of the BV2 reactions of a number of pea varieties.

Evaluation Procedure

The following procedural steps, based upon the aforementioned information and on experience gained over several years in testing numerous pea entries for virus reactions, have proved highly satisfactory for the evaluations of BV2 reactions in pea.

Control Plants. Suitable susceptible (*Mo Mo*) and resistant (*mo mo*) genotypes should be included in each group of entries under test at a given time. Varieties of midseason or late maturity, preferably with dark green foliage and large flat leaves at the first, second, and third true leaf nodes are most desirable because their leaves are less prone to injury from mechanical transmissions and provide a good inoculation surface. Use of early maturing varieties, especially as resistant controls, is undesirable because "thermal isolates" may develop if the incubation period is prolonged to the point of flowering and may confuse evaluations. Perfected Wales (CSC #D100) and

Ranger (GV #581191) were excellent susceptible genotypes; Bonneville (R #66042) was a good resistant genotype. Others may be equally satisfactory.

Number of Plants to Use. The number of plants in each entry in a test is determined by the purpose for which it is included. The potency and character of the virus extract should be verified on 10-12 plants of each control genotype, half at the beginning and half at the end of each transmission. If a given entry is assumed to result from a single plant selection made in the field for mosaic resistance and/or other characters and it is desired only to determine if its progeny is homozygous for the *mo mo* genotype, a minimum of six plants is sufficient at the 0.999 probability level.[9] In reality, a minimum of 15-20 plants is more satisfactory, since this number will also characterize a segregating progeny. The number of plants necessary to determine the purity of an established commercial variety will vary according to the degree of contamination expected as a result of deferred purelining, mechanical mixtures, field crossing, etc.

In this investigation, three fungicide-treated seeds were planted in pasteurized soil contained in a sterilized 4-inch clay pot. The planted pots were maintained at 70°F. until ready for the first inoculation, usually 10-12 days later.

Inoculum. The strain or isolate of the virus and the source of the inoculum are important considerations. Theoretically, any strain or isolate of BV2 predetermined incapable of overcoming the resistance conditioned by the *mo mo* genotype would suffice, but in actuality, some strains are more desirable than others. For example, pea isolate 1[7] was initially used in these tests because it readily produced distinct symptoms on pea. However, it was later discovered to yield "thermal isolates" capable of producing symptoms on the *mo mo* genotype if the inoculated plants were not scored until they reached the flowering stage, a fact that could result in confused evaluations of early maturing entries that flower at the seventh to ninth nodes. Such hazards can be eliminated by using PV2, a strain of BV2 that does not yield "thermal isolates" and which is highly infective, producing a prominent bright mosaic pattern (Fig. 1). Peas were most readily infected, regardless of isolate, when the virus was extracted from pea rather than from some of the other hosts.

Inoculation. Mechanical transmissions should be made twice, initially when the first and second true leaves are fully expanded and again when the third true leaf is fully expanded, about 2-3 days later. Separate controls should be included each time. Leaves are dusted liberally with 400-mesh carborundum, tapped to remove excess powder, and rubbed gently on the upper surfaces with inoculum. Glass spatulas, camel hair or watercolor brushes (sizes 4-8), and cotton swabs can be used to apply inoculum. Leaves are rinsed with water a few minutes after the virus transmissions. A properly inoculated leaf should appear practically unaltered on the surface, and it should never be rubbed so hard that it collapses or becomes necrotic. Leaves of plants grown during the summer months appear to be more resistant to

rubbing injury than those grown during the winter months of low light intensity. Leaves of light green varieties are usually thinner and more easily injured than those of dark green varieties.

Post-Inoculation Temperature and Symptom Expression. Inoculated plants must be maintained at temperatures of 80-85°F. until the plant reactions are finally evaluated. Lower temperatures (70-80°F.) may delay onset of symptoms in the heterozygotes, thus confounding the evaluations. Temperatures below 70°F. may reduce the percentage of infection among the heterozygotes inoculated with some BV2 strains or may even prevent infection with other strains, such as PV2. Within the 80-85°F. range, all susceptible control plants must develop symptoms, usually within 6-8 days after the first transmission, before any reliance can be placed on the reactions of the test entries. If infection of susceptible controls is not complete within 12 days, it is advisable to re-inoculate all healthy appearing plants in the test or to repeat the entire series.

Primary symptoms usually appear on the second or third leaf above the inoculated leaves as a mildly distinct vein clearing, followed by a typical mosaic pattern on the succeeding leaves (Fig. 1).

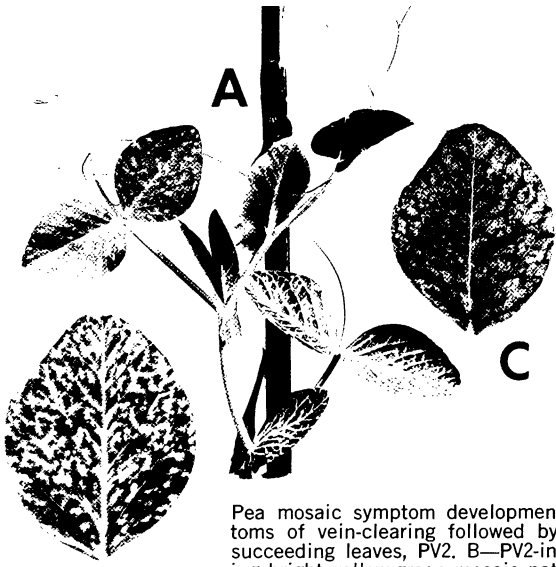

Fig. 1

Pea mosaic symptom development. A—Primary symptoms of vein-clearing followed by mosaic pattern on succeeding leaves, PV2. B—PV2-infected leaflet showing bright yellow-green mosaic pattern. C—Duller light to dark green mosaic pattern incited by BV2 (pea isolate 1).

Test plants remaining healthy when all susceptible controls are diseased are assumed to contain the *mo mo* genotype and thus to be resistant to pea mosaic. If a population is determined to be heterozygous, the healthy plants can be progeny-tested to confirm the *mo mo* genotype and provide the start of a homozygous BV2-resistant line. Plants with symptoms are of the genotypes *Mo Mo* or *Mo mo*. They can be separated by moving all plants to a 65°F. or lower temperature. Symptoms will continue to develop in new growth of the *Mo Mo* genotypes, but not in *Mo mo* genotypes.

Results and Discussion

The reactions of 248 varieties and breeding lines of pea, some of which are listed in table 1, prove the efficacy of the procedure just described. In every set of entries tested, the susceptible controls became completely diseased and resistant controls remained healthy.

With a few exceptions, the test entries were either completely susceptible or homozygous resistant. No attempt was made to determine by temperature manipulations whether any of the completely susceptible entries contained heterozygous individuals, since the original intent was to determine which entries were *mo mo* genotype. Entries with mixed reactions were found after reinoculation of the symptomless plants either to contain some escapes (A-7, B-9, GV-11) or to contain homozygous resistant plants (A4, B23, B24, GV9, GV17, H1, R9, R23, WV2, WV24). Status of the latter group was confirmed by progeny tests of the resistant individuals. Only one entry of a mixed reaction did not fall into either of the above categories (R24). It is conjectured that it was a mechanical mixture or that it may have been infected with a thermal isolate, since additional tests with seed from the same lot indicated *mo mo* genotype. Spot checks of a number of entries (indicated in table 1 by a total number of plants greater than 20) further confirmed the reliability of the procedure.

These tests demonstrate the necessity of testing for genotypes rather than relying on varietal names for disease resistant characters. For example, one entry of Laxton Progress (A47) is *Mo Mo*, but a second (R34) is *mo mo*. Davis Perfection, presumably the original Perfection, was homozygous resistant in these tests (GV33), but McWhorter[11] earlier tested the same variety from a different source and found it heterozygous. Two lots of Elf (A26 and A26A) in these tests were susceptible (*Mo Mo*), but Ford[3] indicated another lot to be free of susceptibles. It is even possible for different lot numbers of the same variety and released by the same company to be of different genotypes, e. g. R9, R23, R24 and R46; R27 and R31.

On the basis of these results it can be said that the only criterion of resistance to BV2 in pea is the occurrence of the *mo mo* genotype. This genotype can be identified and isolated with certainty in the F_2 or later generations with the procedure outlined in this paper.

Table 1.

Reactions of pea varieties to BV2 at a temperature range of 80-85°F.

Entry[1]	Variety and Lot Number	Sus/Inoc[2]
A-3	Pixie (79099)	15/15 s
A-7	Alaska (48001)	15/15 s
A-11	Alaska-7 (49010)	16/16 s
A-14	Yukon (68020)	17/17 s
A-38	XP Alaska-10 (F6116)	15/15 s
A-1	Ea Sweet 11 (96022)	16/16 s
A-17	Ea Sweet 11 (26786)	17/17 s
A-43	Ea Sweet (89019)	13/13 s
A-9	Ea Harvest (68026)	0/16 R
A-18	Ea Harvest (86850)	0/15 R
A-2	Laxton-8 (68034)	12/12 s
A-44	Laxton-8 (88029)	14/14 s
A-21	Sprite (86037)	13/13 s
A-8	Ea Perfn 326 (66056)	0/17 R
A-16	Ea Perfn 326 (46865)	0/15 R
A-13	Perfn 54B (66047)	0/23 R
A-15	W R Perfn (66909)	0/15 R
A-12	Dk Sk Perfn (66004)	0/13 R
A-57	Perfn (63024)	0/20 R
A-4	Fr 640 (66028)	3/13°*h*
A-5	Fr 626 (66020)	0/16 R
A-6	Fr 69 (66044)	14/14 s
A-10	Fr 37 (66012)	14/14 s
A-55	Fr 37 (Freedom) (96037)	12/12 s
A-56	Fr 650 (46026)	0/12 R
A-22	Signet (R611111)	0/13 R
A-26	Elf (84049-2)	15/15 s
A-26A	Elf (76036)	15/15 s
A-27	Laurel (84060)	15/15 s
A-36	Canner Prince (84089)	0/12 R
A-41	Canner Prince (84047)	0/13 R
A-42	Green Bay (84062)	15/15 s
A-58	Canner King (76003)	0/25 R
A-40	Trojan (F6214)	0/20 R
A-39	Nugget (16057)	18/18 s
A-45	Little Marvel (96053)	0/14 R
A-46	World's Record (75121)	17/17 s
A-47	Laxt. Progress (53753)	33/33 s
A-48	Hundredfold (97090-1)	0/13 R
A-49	Rondo (27065)	0/17 R
A-50	Progress #9 (97098-2)	0/12 R
A-51	Premium Gem (66003)	0/12 R
A-52	Asgrow 40 (27067-1)	17/17 s
A-53	Giant Stride (7162)	19/19 s
B-7	Alaska 14-1 (C3)	17/17 s
B-9	Late Alaska (C4)	17/17 s
B-24	Late Alaska (C5)	10/15°*h*
B-8	Allsweet AA15 (C13)	18/18 s
B-11	Imp. Surprise (C12)	0/9 R

Entry[1]	Variety and Lot Number	Sus/Inoc[2]
B-1	Perfn 42 (C54)	0/9 R
B-4	Perfn 25 (C57)	0/11 R
B-2	Perfn Fr 70 (F28)	0/9 R
B-5	Erickson Perfn (C37)	0/12 R
B-19	Fraser	0/11 R
B-6	Multifreezer (F3)	0/12 R
B-15	Sm Sieve Fr	0/12 R
B-20	Midfreezer	15/15 s
B-23	GSM Canner	10/14°*h*
CM-2	Lilalaska (M-50201)	14/14 s
CM-4	Sm Sv Alaska (M-50531)	17/17 s
CM-5	Ea Sweet A45 (M-51202)	0/12 R
CM-6	A-45 (612005)	0/15 R
CM-1	Fr 259 (M-55902)	0/15 R
CM-3	Sm Sv Fr (M-56302)	0/14 R
CSC-3	Ea Perfn (3502)	0/24 R
CSC-4	Ea Perfn (3040)	0/21 R
CSC-5	Ea Perfn (3400)	0/17 R
CSC-6	Ea Perfn (3019)	0/24 R
CSC-9	Ea Perfn (3425)	0/20 R
CSC-7	Perfn (5072)	0/20 R
CSC-11	Lg Seeded Perfn (2731)	16/16 s
CSC-1	Perfn Fr (3913)	0/21 R
CSC-8	Perfn Fr 39	0/19 R
CSC-2	Freezer (3324)	0/14 R
CSC-10	Freezer (5147)	0/19 R
CSC-12	Imp Perfd Wales (D100)	349/349 s
CSC-12	Imp Perfd Wales (3201)	0/18 R
CSC-18	Perfd Wales (D100)	349/349 s
FM-1	Pacific Perfn (F33)	0/16 R
FM-2	Miragreen (F27)	15/15 s
FM-3	Giant Stride	11/11 s
GV-19	Late Alaska (58684)	13/13 s
GV-20	Sm Sv Alaska (51025)	12/12 s
GV-20A	Sm Sv Alaska (51201)	20/20 s
GV-21	W R Alaska (61010)	12/12 s
GV-44	Alaska (54201)	20/20 s
GV-37	Ea Sweet 11 (53206)	20/20 s
GV-36	W R Surprise (59A201)	0/20 R
GV-39	W R T. Laxton (91149)	12/12 s
GV-42	Rocket (58A202)	17/17 s
GV-34	Ea Perfn (109-36201)	0/17 R
GV-3	Ea Perfn 174 (591120)	0/18 R
GV-3A	Ea Perfn 174 (74201)	0/20 R
GV-14	Ea Perfn 173 (581195)	0/15 R
GV-38	W R Perfn (35201)	0/17 R
GV-41	Dk Sk Perfn (750201)	0/20 R
GV-33	Davis Perfn (62347)	0/35 R
GV-35	Perfd Fr 60 (76201)	0/20 R
GV-10	Jade (20005)	0/16 R
GV-13	Cascade (56-1181)	0/16 R
GV-13A	Cascade (34-b-202)	0/20 R
GV-5	Midway (55-631)	0/16 R

Entry[1]	Variety and Lot Number	Sus/Inoc[2]
GV-5A	Midway (32202)	0/20 R
GV-6	Pride (55-608)	0/15 R
GV-6A	Pride (39-A-202)	0/20 R
GV-16	Bridger C42 (55-615)	0/16 R
GV-16A	Bridger (33-F-203)	0/20 R
GV-7	Hyalite (55-710)	0/14 R
GV-40	N W R Hyalite (71201)	0/20 R
GV-9	Ea Freezer (601128)	5/14°*h*
GV-18	Ea Freezer (61461)	0/15 R
GV-15	Ea Freezer 163 (591122)	0/17 R
GV-17	M P Lt Freezer (601138)	3/10°*h*
GV-1	Viking (25003)	0/17 R
GV-1A	Viking (25206)	0/20 R
GV-8	Ranger (55-661)	31/31 s
GV-8A	Ranger (581191)	124/124 s
GV-12	Climax (56-520)	0/14 R
GV-43	Jordan (62222)	20/20 s
GV-11	Alderman (50-584)	12/12 s
GB-1	Dwarf Telephone (1349)	14/14 s
GB-2	Progress #9 (1535)	0/13 R
GB-3	Pacific Market (2038)	13/13 s
GB-4	Morse's 55 (7007)	0/12 R
H-4	Wando (1355)	0/12 R
H-1	Frosty (1349)	5/14°*h*
H-6	Little Marvel (1346)	0/16 R
H-7	World Record (1354)	10/10 s
H-3	Lincoln (1357)	16/16 s
H-2	Midseason Giant (1353)	14/14 s
H-5	Greater Progress (1345)	0/13 R
R-6	Alaska 423 (61063)	17/17 s
R-7	Sm Sv Alaska (61079)	18/18 s
R-28	Alaska Fr (61454)	16/16s
R-32	Ea Sweet 11 (10024)	17/17 s
R-1	Surprise (74082)	0/15 R
R-5	Ace (64082)	0/15 R
R-21	Perfn (61015)	0/14 R
R-22	Perfn New Line (51031)	0/23 R
R-42	Perfn New Line (31037)	0/25 R
R-43	Bonneville (66042)	0/415 R
R-2	Shoshone	0/14 R
R-4	Perfd Fr (61514)	0/15 R
R-10	Eureka (61006)	0/20 R
R-41	Famous (71004)	0/30 R
R-12	Midfreezer (61057)	15/15 s
R-9	Frosty (02005)	4/14°*h*
R-23	Frosty (61357)	11/15°*h*
R-24	Frosty (61358)	2/35 R
R-46	Frosty (12712)	3/15°*h*
R-40	Early Frosty (02006)	0/25 R
R-47	Early Frosty (32017)	0/20 R
R-45	Early Frosty (12012)	0/20 R
R-3	Sm Sv Freezer (61009)	0/16 R
R-11	Ea Freezer (601261)	14/14 s
R-27	Early Canner (61506)	30/30 s

Entry[1]	Variety and Lot Number	Sus/Inoc[2]
R-31	Early Canner (61508)	0/30 R
R-34	Laxt. Progress (82753)	0/38 R
R-8	Alderman (41043)	12/12 s
R-33	Icer (82009)	9/9 s
R-35	Laxt. Superb (82721)	0/21 R
R-36	Freezonian (84739)	9/9 s
R-37	Lincoln (81739)	9/9 s
R-44	Little Marvel (22005)	0/20 R
WV-22	Sm Sv Alaska (19046)	20/20 s
WV-23	Alaska (49214)	18/18 s
WV-30	W R Alaska (32202)	18/18 s
WV-26	Ea Sweet AA15 (58229)	20/20 s
WV-35	Ea Sweet A45 (0219)	0/18 R
WV-19	W R T. Laxton (23271)	20/20 s
WV-18	Gr Sd T. Laxton (51279)	16/16 s
WV-27	Surprise (24562)	0/15 R
WV-20	Sm Sd T. Laxton (21272)	16/16 s
WV-28	Ea Perfn (48246)	0/20 R
WV-29	Dk Gr Perfn (62236)	0/20 R
WV-3	Scout (9233)	18/18 s
WV-36	Scout (3314)	20/20 s
WV-34	Chinook (64226)	20/20 s
WV-2	Freez Elite (14927)	8/13°*h*
WV-24	Freez Elite (14243)	8/16°*h*
WV-1	Valley Gem (17748)	11/11 s

[1] Seed Sources:

A—Asgrow Seed Co., New Haven, Conn.
B—W. Brotherton Seed Co., Moses Lake, Washington
CM—Crites Moscow Growers Inc., Moscow, Idaho
CSC—Canners Seed Corp., Lewisville, Idaho
FM—Ferry-Morse Seed Co., Mountain View, Calif.
GV—Gallatin Valley Seed Co., Twin Falls, Idaho
GB—Gill Bros. Seed Co., Portland, Oregon
H—Joseph Harris Company, Inc., Rochester 11, New York
R—Rogers Bros. Seed Co., Twin Falls, Idaho
WV—Western Valley Seed Co., Lewiston, Idaho

Abbreviations:

Ea—early; Dk—dark; Sk—skin; Perfn—Perfection; Perfd—Perfected; W R—wilt resistant; Fr—Freezer; Sm—small; Sv—sieve; N W R—near wilt resistant; Gr—green; Sd—seeded.

[2] Sus/Inoc—number susceptible/number inoculated; s—susceptible; R—resistant (*mo mo genotype*); *h*—heterozygous—carrying plants with *mo mo* but also some susceptibles. °—indicates lines re-inoculated and survivors progeny tested to confirm *mo mo* genotype.

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