Breeding for resistance to diseases in lettuce: successes and challenges

Edward J. Ryder[1], Rebecca C. Grube[1], Krishna V. Subbarao[2], and Stephen T. Koike[3]
[1]United States Department of Agriculture-Agricultural Research Service, 1636 E. Alisal St., Salinas, California 93905, USA
[2]Department of Plant Pathology, University of California, Davis, 1636 E. Alisal St., Salinas, California 93905, USA
[3]University of California Cooperative Extension, Monterey County, 1432 Abbott St., Salinas, California, 93901, USA
Contact: eryder@pw.ars.usda.gov, (+1) 831 755 2860.

Abstract: Lettuce mosaic has been a long-term world wide problem. Resistance is available at a single locus. A single gene, partially dominant, for mild reaction, can be combined with an allele for resistance to produce a higher level of resistance. Breeding work is under way. An additional source of high level resistance has been tentatively identified. A new seed borne strain of LMV that overcomes resistance may become a problem. Big vein has also been a long-term problem. Moderate sources of resistance have been identified, based upon proportion of plants showing symptoms at harvest time. Pavane appears to have the highest level of the identified sources. The species *Lactuca virosa* appears to have complete resistance and is being used in a breeding program. Verticillium is a new disease in lettuce. No iceberg type lettuce cultivars tested are resistant. Several other cultivars of different type are resistant and are being used in breeding.

Keywords: lettuce, *Lactuca sativa, Myzus persicae, Lactuca virosa, Lactuca serriola, Nasonovia ribisnigri, Olpidium brassicae*, *Verticillium dahliae*, lettuce mosaic, big vein, Verticillium wilt

Introduction

The Vegetable Breeding group at the US Agricultural Research Station in Salinas, California has a large varied program dealing with disease, insect and stress resistance, and other problems, of lettuce, spinach and melons. In the lettuce resistance part of the program, we deal with 15 different diseases, pests and disorders as they affect five different types of lettuce (crisphead, romaine, green leaf, red leaf, and butterhead). In this paper, I will discuss work with three diseases of lettuce: lettuce mosaic (LM), big vein (BV) and Verticillium wilt (Vert).

Lettuce mosaic

First identified over 80 years ago (Jagger 1921), LM became a world wide, serious economic problem, largely due to its seedborne transmission characteristic (Newhall 1923). It is passed from plant to plant and from field to field primarily by the green peach aphid (*Myzus persicae*). It is controlled in two ways: planting seeds virtually free of virus, and by use of resistant cultivars. Resistance is controlled by either of two recessive alleles at a single locus (*Momo*^{g} or *Momo*^{e}) (Bannerot et al. 1969, Ryder 1970. Ryder 2002).

Resistance is based on a single gene and may be considered vulnerable. New strains of the virus appear at intervals. Most seem to be restricted to certain areas or may disappear quickly. However, in recent years, several strains have persisted for longer periods of time, and at least two can overcome the resistance conferred by one or the other or both alleles. Therefore the search for additional sources of resistance has continued. In a 1988 screening test we found a stem lettuce from Egypt (Balady Aswan, BA) that consisted of a mixture of two types, one with anthocyanin (BAR) and one without (BAG). BAR was resistant and reacted in the same way as the alleles previously identified. BAG was infected systemically by the virus but with a much reduced symptom expression, designated mild. The reaction was controlled by a single partially dominant gene, *mild* (*Mi'Mi*) (Table 1) (Ryder 2002). When crossed to resistant parents, four phenotypes appeared in the F_2: mild (two subtypes because of the partial dominance), mottled (susceptible), no symptom (NS), and resistant, in the ratio 9:3:3:1(Table 2). The NS type was confirmed in F_3 families. NS plants were *momoMi'Mi'*. Subsequent work showed that NS plants were not always symptom free (Table 3), but were consistently more resistant than the resistant type (*momo*). A lower percentage of plants showed symptoms compared to resistant, mild, and susceptible plants. The number of days to symptom expression was greater than for the other three types.

Table 1. Segregation for mosaic reaction type in a cross between Balady Aswan Green (BAG) (mild) and Salinas (severe).

Population	Observed			X^2	p
	Mild	Inter.	Sev.		
F_2 (1:2:1)	35	63	33	0.19	0.50-0.70
	Mild	Seg.	Sev.		
Among F_3 families (1:2:1)	35	70	23	3.37	0.10-0.20
Within seg. F_3 families (3:1)	242[1]		67	1.73	0.10-0.20

[1] Mild and intermediate combined

Table 2. Segregation for mosaic reaction type in a cross between Balady Aswan Green (BAG) (mild) and PI 251245 (resistant). Observed numbers.

Population	Mild	Sev.	NS	Res.	X^2	p
F_2 (9:3:3:1)	106[1]	38	34	13	0.35	0.95-0.98

[1] Mild and intermediate combined

For breeding purposes, we crossed a highly resistant line with two resistant cultivars, Salinas 88 and Vanguard 75, and have selected for good crisphead heading type as well as high level resistance. We have also backcrossed to Salinas 88 to further improve heading type.

Recently, we screened our collection of Plant Introduction (PI) accessions to look for additional sources of LM resistance and identified 10 with resistance. Most crosses with resistant and susceptible parents indicated the presence of the two alleles previously identified. Several are still being investigated. One accession, PI 226514, was separated into two forms, with anthocyanin (tinged, tn), and without anthocyanin (green, gr). An F_2 of the cross 226514tn x Salinas segregated 33 mottled:11 resistant:4 NS. F_3 families from mottled F_2 plants segregated 1 all mottled : 2 segregating, and F_3 families from resistant F_2 plants produced all resistant plants, as expected. Two F_3 families from three of the NS plants produced resistant and NS plants, while one F_3 family produced all NS plants. F_4 families from four plants of this last family produced all NS plants. A second planting of one of the F_4

families again produced all NS plants. This is an unexpected result and we are continuing to investigate.

Table 3. Comparison of four reaction types (G-5-1, highly resistant; Balady Aswan Red (BAR), resistant; Balady Aswan Green (BAG), mild; Salinas, severe) in three experiments at different seasons.

Entry	G-5-1	BAR	BAG	Salinas
Inoculated 19 December				
No. plants	24	26	29	30
Percent infected	45.8	61.5	89.7	100
Mean days to symptom	37.6	32.6	20.0	14.0
Inoculated 20 March				
No. plants	12	12	12	12
Percent infected	25.0	66.7	83.3	91.7
Mean days to symptom	33.0	18.9	13.0	10.8
Inoculated 21 August				
No. plants	12	12	11	12
Percent infected	8.0	50.0	100	83.3
Mean days to symptom	34.0	29.0	13.5	8.3

Regarding the new isolates of LM, an investigation (Krause-Sakate et al. 2002) identified one group, LMV-Most, which overcomes the resistance of both *mo* alleles and is also transmitted through the seed. This is the first highly virulent strain with the seed transmission characteristic. LMV-Most isolates have been found in Europe, North Africa, South America, and in a number of commercial seed lots. Such widespread occurrence, and the presence in seed lots, strongly suggests further dispersal and a potential threat to lettuce crops everywhere. The higher level resistances described above are overcome by LMV-Most (Candresse, Maisonneuve, personal communication).

Big vein

Big vein in lettuce was first identified in California (Jagger and Chandler 1934) and has since become a problem in most lettuce production areas. It is a virus disease introduced into lettuce roots by a fungus, *Olpidium brassicae*. The nature and identity of the virus has been under review for many years. The latest candidate is Mirafiori lettuce virus, and it has been shown by mechanical transmission to consistently cause big vein symptoms in lettuce (Roggero et al. 2000, Lot et al. 2002).

Studies by Westerlund et al. (1978a, 1978b) and Roggero et al. (2000) show that plants infected with virus may not show symptoms. Since the vector is always present in the soil where big vein occurs, one can assume either that all plants in a big vein prone field have big vein but may not show symptoms or that the fungus does not infect under certain conditions. The development of big vein resistant cultivars, therefore, is based upon the proportion of plants that show symptoms at a given time, usually at harvest. Vein clearing, leaf stiffening, and poor head development affect the quality of the lettuce, and a plant that can delay the onset of these symptoms can be considered to be resistant.

We have screened over 1000 cultivars and 560 PI accessions and have identified several items defined as resistant based upon the above criterion. These include over 40 cultivars and PI accessions in *L. sativa* and *L. serriola*. The Latin cultivar Pavane has consistently shown a higher level of resistance than other cultivars. We crossed Pavane with Pacific, one of our own crisphead releases, backcrossed a derived line with Pacific for improved heading type, and have identified several lines with resistance superior to Pacific and equivalent to Pavane (Table 4). This result suggests that we have successfully transferred resistance alleles from Pavane to a Pacific type.

Table 4. Comparison for big vein reaction of nine BCF_4 families, $Pacific^2$ x Pavane, with their parents and a susceptible check. Greenhouse test with three replications.

Family	% Big vein	Family	% Big vein
01-2058	33.3	01-2062	50.0
01-2060	38.9	01-2064	50.0
01-2061	44.4	01-2059	61.1
01-2063	44.4	Pacific	63.9
01-2057	47.2	01-2065	75.0
Pavane	47.2	Great Lakes 65	94.3

Bos and Huijberts (1990) showed that two accessions of *L. virosa* showed no symptoms of disease five weeks after inoculation. We tested one of those accessions (IVT 280) and an additional 18 of *L. virosa* and also found no symptoms. This suggests a high level of resistance in the species itself. IVT 280 has been used as a source of resistance to two other lettuce problems, beet western yellows and the lettuce aphid (*Nasonovia ribisnigri*) (Maisonneuve et al. 1991, van der Arend et al. 1999). We have obtained from Dr. Maisonneuve early generation backcross breeding lines from various crosses with IVT 280. We are now investigating the inheritance and breeding potential for big vein resistance of this material. If this form of BV resistance can be transferred to cultivated lettuce, we will select for resistance in crisphead and romaine types.

Verticillium wilt

Verticillium dahliae is one of two *Verticillium* species causing important diseases affecting a range of crops. This species was identified in 1995 as the cause of Verticillium wilt (Vert) in lettuce, in a field near Watsonville, California (Bhat and Subbarao 1999). The disease subsequently spread to other fields in the vicinity and also into the much larger and more important lettuce production area, the Salinas Valley. It is likely to continue spreading, and it causes severe damage, particularly to crisphead lettuce, which occupies most of the lettuce growing area in coastal California. Consequently, it is likely to become a serious economic problem. The fungus causes green to black vascular discoloration in the roots and wilting and yellowing of lower leaves. On mature heads of iceberg lettuce, large numbers of microsclerotia form, and the head becomes shrunken and desiccated (Subbarao et al. 1997).

At present, the most effective control is fumigation with methyl bromide. This is an expensive treatment, and its use will be phased out in 2005. Rotation with other crops can be useful, but the number of possible crop combinations is limited. It is most likely that the use of resistant cultivars will be the most useful control.

We began a cooperative field screening program in 1996 to identify sources of resistance to Vert. Plants were rated by inspection of the taproots for presence of vascular discoloration and distance of the discoloration from the crown. Plants showing no discoloration were considered resistant. Plants with discoloration that reached the crown were rated as susceptible. Plants with discoloration short of the crown were rated as possibly resistant. Every iceberg type cultivar tested was found to be susceptible. We also screened other types of cultivars and a few PI accessions and found resistance in red and green leaf, butterhead, Latin, stem, and batavia types. No Latin type tested was susceptible (Table 5). The cultivars were rated on the basis of at least two field trials. Several cultivars gave inconsistent results in different trials and are not listed.

Table 5. Results of field tests for resistance to Verticillium wilt. Selected cultivars of various types.

Susceptible	
	Iceberg- Salinas, Bronco, Pacific, Glacier, Calmar, Tiber, Target, Malika
	Batavia- Avoncrisp, Holborn Standard
	Romaine- Darkland, Green Towers
	Leaf- Prizehead, Waldmann's Green, Ruby
	Butterhead- Summer Bibb
	Latin- None
	Stem- Celtuce
Resistant	
	Iceberg- None
	Batavia- La Brillante
	Romaine- Lobjoits
	Leaf- Australian, Red Grenoble, Parsberg
	Butterhead- Margarita, Tania, Ostinata
	Latin- Little Gem, Gallega
	Stem- Balady Aswan, Balady Behera, Balady Banha

We make it a practice to keep remnant seed of all F_2 populations and many F_3 family groups in our freezer storage facility. Many of the cultivars we identified as resistant had already been crossed to iceberg and romaine lettuce cultivars for other disease resistance studies or for genetic studies. We were therefore able to begin the selection phase of a breeding program immediately. For example, we had in storage F_3 families of the cross Pacific (iceberg, susceptible) x La Brillante (batavia, resistant). We now have F_6 lines and BCF_3 families that are approaching commercial type. In addition we have F_3 and F_4 families from seven other crosses between susceptible and resistant cultivars.

The breeding program is being hampered slightly by a paucity of Vert infested fields that growers are willing to delay fumigating long enough to permit a resistance screening test. Therefore, we sometimes have to alternate resistance screening in infested fields with plantings for selection in healthy fields. We also do not have a clear picture of the etiology of the disease or of the nature or inheritance of resistance. We have had difficulty developing a reliable controlled environment screening and testing procedure, primarily because of false negatives for susceptible materials and apparently false positives for resistant materials. Development of a useful procedure is a top priority.

So to recapitulate the title of this presentation, we have had successes in each of these programs. Hopefully, I have made it clear that there are still some important challenges.

Acknowledgements

We wish to thank the California Lettuce Research Board for support of parts of the research. Thanks to Bert Robinson, Dave Milligan, JoAnn Tanaka, Ravi Bhat, and Rami Aburomia for technical support.

References

Bannerot, H., Boulidard, L., Marrou, J. and Duteil, M. 1969. Study of the inheritance of tolerance to lettuce mosaic virus in the variety Gallega de Invierno. Annales Phytopathologie 1: 219-226.

Bhat, R.G. and Subbarao, K.V. 1999. Host range specificity in *Verticillium dahliae*. Phytopathology 89: 1218-1225.

Bos, L. and Huijberts, N. 1990. Screening for resistance to big-vein disease of lettuce (*Lactuca sativa*). Crop Protection 9: 446-452.

Jagger, I.C. 1921. A transmissible mosaic disease of lettuce. Journal of Agricultural Research 20: 737-741.

Jagger, I.C. and Chandler, N. 1934. Big vein, a disease of lettuce. Phytopathology 24: 1253-1256.

Krause-Sakate, R., Le Gall, O., Fakhfakh, H., Peypelut, M., Marrakchi, M., Varveri, C., Pavan, M.A., Souche, S., Lot, H., Zerbini, F.M. and Candresse, T. 2002. Molecular and biological characterization of lettuce mosaic virus (LMV) isolates reveals a distinct and widespread type of resistance-breaking isolate: LMV-Most. Phytopathology 92: 563-572.

Lot, H., Campbell, R.N., Souche, S., Milne, R.G. and Roggero, P. 2002. Transmission by *Olpidium brassicae* of Mirafiori lettuce virus and lettuce big vein virus, and their roles in lettuce big-vein etiology. Phytopathology 92: 288-293.

Maisonneuve, B., Chovelon, V. and Lot, H. 1991. Inheritance of resistance to beet western yellows virus in *Lactuca virosa* L. HortScience: 26: 1543-1545.

Newhall, A.G. 1923. Seed transmission of lettuce mosaic. Phytopathology 13, 104-106.

Roggero, P., Ciuffo, M., Vaira, A.M., Accotto, G.P., Masenga, V. and Milne, R.G. 2000. An *Ophiovirus* isolated from lettuce with big-vein symptoms. Arch. Virol. 145: 2629-2642.

Ryder, E. J. 1970. Inheritance of resistance to common lettuce mosaic. J. Amer. Soc. Hort. Sci. 95: 378-379.

Ryder, E. J. 2002. A mild systemic reaction to lettuce mosaic virus in lettuce (*Lactuca sativa*): Inheritance and interaction with an allele for resistance. J. Amer. Soc. Hort. Sci. 127: 814-818.

Subbarao, K.V., Hubbard, J.C., Greathead, A. and Spencer, G.A. 1997. Verticillium wilt. In: Davis, R.M., Subbarao, K.V., Raid, R.N., and Kurtz, E.A. (eds.), Compendium of Lettuce Diseases, APS Press, St. Paul. pp 26-27.

Van der Arend, A.J.M., Ester, A., Schijndel, J.T. van 1999. Developing an aphid resistant butterhead lettuce "Dynamite". Eucarpia Leafy Vegetables 99. Palacky University, Olomouc, Czech Republic. pp. 149-158.

Westerlund, F.V., Campbell, R. N. and Grogan, R.G. 1978a. Effect of temperature on transmission, translocation, and persistence of the lettuce big-vein agent and big-vein symptom expression. Phytopathology 68: 921-926.

Westerlund, F.V., Campbell, R.N., Grogan, R.G. and Duniway, J.M. 1978b. Soil factors affecting the reproduction and survival of Olpidium brassicae and its transmission of big-vein agent to lettuce. Phytopathology 68: 927-935.