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## Report of the Berry Working Group (Strawberry, Raspberry/ Blackberry, Blueberry)

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# REPORT OF THE BERRY WORKING GROUP<sup>1</sup>

## (Strawberry, Raspberry/Blackberry, Blueberry)

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### STRAWBERRY (*FRAGARIA*)

Strawberries are a relatively recently domesticated crop. The most commonly cultivated strawberry, *Fragaria x ananassa*, is a hybrid of the North American *F. virginiana* and the South American *F. chiloensis* (Maas 1998). These parental species are still grown in some areas and *F. virginiana* is the primary wild, sexually compatible relative to the cultivated strawberry. In addition to *F. virginiana*, *F. vesca* and its subspecies are also present in the United States. *Fragaria x ananassa* and *F. virginiana* readily cross. Introgression of pest resistance traits into the wild strawberry population is likely, as substantial amounts of crop-weed introgression has already occurred throughout the midwest, northeast, and southeast United States (Jim Hancock pers. comm.). Introgression has occurred to the extent that it is difficult to find “pure” populations of *Fragaria virginiana* in many areas. Strawberries suffer from several limiting diseases, insects, nematodes, and weed problems (Table 1).

In addition to those listed above, resistance to root-lesion nematode (Potter and Dale 1994), *Phytophthora cactorum*, *Sphaerotheca macularis*, and strawberry aphids (Shanks and Moore 1995) have been identified in cultivars of strawberry. Much of the pest resistance that has been incorporated into strawberry breeding programs has been derived from wild relatives. A substantial effort has been targeted specifically towards *Phytophthora fragariae* resistance (van

de Weg *et al.* 1997), but in most breeding programs, elite types have been screened after selection for other horticulturally important traits. New efforts to breed for resistance to *P. fragariae* and *P. cactorum* are underway (Maas *et al.* 1993).

Strawberry is considered to be relatively amenable to transformation using *Agrobacterium* (Nehra *et al.* 1992), and the technique is being used to genetically engineer virus resistance. Strawberry plants have been transformed using the coat protein gene from strawberry mild yellow edge virus (Finstad and Martin 1995), and resulting plants are being evaluated. Other traits that are currently under investigation include glyphosate resistance, broad-spectrum fungal resistance through the use of the stilbene synthase gene and genes for systemic acquired resistance, and nematode resistance through the use of transgenes producing protease inhibitors (Morgan and Guttererson 1998).

Although sexually compatible relatives to strawberry are found in the United States, they are not considered to be a weed problem in strawberry fields. Cultivated strawberries are not capable of persisting outside the area of cultivation in the California production system, but have been found to escape in many areas of the midwestern and southern United States. Strawberries lack significant weedy characteristics and the addition of pest resistance would be unlikely to substantially increase the crop’s ability to persist.

<sup>1</sup> Group Report from the “Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems,” in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

**Table 1. Strawberry pests and status of resistance in cultivated and wild species.<sup>1</sup>**

<u>Strawberry Pest</u>	<u>Status of Resistance in Cultivated Varieties</u>	<u>Resistance in Wild Relatives</u>
Angular leaf spot ( <i>Xanthomonas fragariae</i> <sup>2</sup> )	Some cultivars with resistance	<i>F. moschata</i> tolerant. <i>F. virginiana</i> and <i>F. vesca</i> moderate resistance (Maas 1998; Maas, Pooler, and Galletta 1995)
Leaf scorch ( <i>Diplocarpon earlianum</i> <sup>2</sup> )	No commercial resistance; Resistance in wild relatives and some non-horticultural varieties (Xue <i>et al.</i> 1996)	Not reported
Leaf spot ( <i>Mycosphaerella fragariae</i> <sup>2</sup> )	Commercial cultivars with resistance (Darrow 1962; Horn, Burnside, and Carver 1972; Nemeč 1971)	Not reported
Botrytis fruit rot ( <i>Botrytis cinerea</i> <sup>2</sup> )	<sup>3</sup> Engineered resistance; Resistant cultivars	Not reported
Anthracnose ( <i>Colletotrichum</i> spp. <sup>2</sup> )	Resistant cultivars through directed breeding (Olcott-Reid and Moore 1995)	Not reported
Red stele root rot ( <i>Phytophthora fragariae</i> var. <i>fragariae</i> <sup>2</sup> )	<sup>3</sup> Resistance genes identified to races 1 and 2; Resistant cultivars through directed breeding (Scott <i>et al.</i> 1976; 1984)	<i>F. virginiana</i> (Gooding 1973) <i>F. chiloensis</i> (Galletta <i>et al.</i> 1994)
Verticillium wilt ( <i>Verticillium</i> spp. <sup>2</sup> )	<sup>3</sup> Engineered resistance; Moderate resistance in some cultivars (Shaw <i>et al.</i> 1997)	<i>F. chiloensis</i> (Shaw <i>et al.</i> 1996)
Black root rot ( <i>Pythium ultimum</i> , <i>Rhizoctonia fragariae</i> , and <i>Pratylenchus</i> spp. <sup>2</sup> )	<sup>3</sup> Engineered resistance to <i>Pythium</i> spp.; Moderate, regional resistance in some cultivars (Wing <i>et al.</i> 1995)	Not reported
Strawberry mottle virus	<sup>3</sup> Coat protein mediated resistance	Not reported
Strawberry mild yellow edge virus	<sup>3</sup> Coat protein mediated resistance	Not reported
<i>Pratylenchus</i> , <i>Aphelenchoides</i> , <i>Xiphinema</i> , <i>Belonolaimus</i> , <i>Meloidogyne</i> <sup>2</sup>	<sup>3</sup> Protease inhibitor transgenes. Tolerance in some cultivars	<i>F. chiloensis</i> and <i>F. virginiana</i> (Potter and Dale 1994)
Spider mite ( <i>Tetranychus urticae</i> <sup>2</sup> )	Cultivar resistance (Easterbrook and Simpson 1998)	<i>F. chiloensis</i> and <i>F. virginiana</i> (Shanks and Moore 1995; Easterbrook and Simpson 1998)
Lygus bugs ( <i>Lygus lineolaris</i> <sup>2</sup> )	Cultivar resistance	<i>F. virginiana</i> and <i>F. chiloensis</i> (Maas 1998)
Bud weevil ( <i>Anthonomus signatus</i> )	No commercial resistance	Not reported
Flower thrips ( <i>Franliniella</i> spp)	No commercial resistance	Not reported
Sap beetles ( <i>Stelidota geminata</i> )	No commercial resistance	Not reported
Root weevils ( <i>Otiorynchus</i> spp)	No commercial resistance	Not reported
Weeds	<sup>3</sup> Round-Up Ready	None

<sup>1</sup>Farr *et al.* 1989; additional information compiled from Maas 1998<sup>2</sup>Pest occurs on wild relatives, resistance derived from or identified in wild populations<sup>3</sup>Under consideration or in development

## WHAT IS NEEDED?

The impact that pest populations have on the spread of wild, sexually compatible relatives is not clear. It is assumed that the environment limits the growth and spread of strawberries more than pest pressure. Little evidence of disease has been noted on the leaves and fruit of natural populations, but the root pathogens of wild strawberry have not been characterized at all; therefore broad-spectrum resistance to fungal plant pathogens may or may not provide an advantage. Information is also lacking on the competitive interactions within natural communities, and we do not know if engineered traits could make the strawberry a more effective competitor within its natural community. For example, even if the insertion of a pest resistance gene did not cause wild strawberry to be a significant weed problem in agriculture, are there endangered plants that might be replaced by a more aggressive strawberry? This information could minimize any concerns that the environmental community might voice about minimizing diversity in native plant populations. It would be helpful to determine if broad-spectrum resistance to fungal plant pathogens already occurs in native species.

Experiments are currently underway in which *Fragaria chiloensis* and *F. virginiana* growth is being compared in methyl bromide fumigated and non-fumigated soils. This will provide information concerning general pest resistance in the native species. Lists of endangered species and primary locations are maintained by various government agencies. These could provide information concerning the co-existence of wild strawberry relatives and endangered native species.

Strawberry producers have used pest resistance genes incorporated through conventional breeding for several decades. These resistant cultivars have been grown over large acreages for long periods of time, but increased weediness of strawberry has not been observed. There does not appear to be any evidence that there should be concern about the introduction of pest resistance genes in this crop, particularly those that are specific to a single pathogen.

## RASPBERRY/BLACKBERRY (*RUBUS*)

Cultivated raspberries and blackberries are a diverse group. Most species have perennial root systems and biennial canes; however, some produce perennial canes, and others annual canes. Species producing edible raspberries that are used commercially include *R. idaeus* subsp. *vulgatus*, *R. idaeus* subsp. *strigosus*, *R. occidentalis*, and *R. glauca*. Commercial blackberries are most commonly in the subgenus *R. eubatus*. Hybrids between blackberries and raspberries are also commonly grown. Several *Rubus* species are found wild within the United States. Crosses within each subgenus are common and crosses between the subgenera are viable at higher ploidy levels. Diploid hybrids between *R. subg. Ideobatus* and *R. subg. Eubatus* are usually sterile.

Raspberries suffer from a variety of diseases, the most important of which are summarized in Table 2. Wild brambles are a problem weed in raspberry and blackberry production. Cultivated bramble primocanes are controlled in some areas using herbicide application. This practice accounts for a large portion of the weed management as well. In addition, weed control between rows is achieved through clean tilling, mulch, and herbicide application. Herbicides that are used to control *Rubus* spp. include imazapyr, sulfometuron-methyl, glyphosate, tebuthiuron, picloram, and hexazinone. Genes for resistance to pests, such as aphid and raspberry bushy dwarf virus resistance, were incorporated into raspberry cultivars and have been used since the 1940's. There is no evidence that these traits have caused an increase in the weediness of the species grown as crops. Red raspberry is not a weedy plant in areas where it has been grown commercially since the 1920's. There is also no evidence that these resistance traits have conferred any advantage to wild populations.

Most of the diseases that occur on cultivated *Rubus* spp. are likely to occur on the native wild relatives as well (Farr *et al.* 1989). Accordingly, most of the resistance genes that have been incorporated into commercially-grown species have been derived from wild relatives. Since the resistance genes introduced through breeding efforts have come from native species, there is a

high degree of familiarity with the traits that are being used in genetic engineering. However, there is the possibility that genes conferring broad-spectrum resistance could contribute to weediness of native species, but this risk is difficult to assess because little information exists concerning the impact that pathogen complexes have on wild relatives.

Introgression of pest resistance traits into wild *Rubus* populations is very likely and has occurred where commercial varieties are the same species as the native *Rubus*. In areas where different species exist together, introgression is also likely to occur, but at a slower rate. The consequences of pest resistance genes moving into the native species are considered to be of minimal risk in cases in which similar resistance phenotypes already occur in native species. Herbicide resistance would not be recommended.

#### WHAT IS NEEDED?

An extensive literature search on the occurrence of pathogens and pests on native species would contribute significantly to determining the impact of broad-spectrum resistance genes. For example, it would be important to determine if the

Himalaya berry (*R. porcerus*) is sexually compatible with native and commercial *Rubus* spp.. While the majority of diseases found on cultivated red raspberry (*R. idaeus*) and blackcap (*R. occidentalis*) also occur on wild relatives, no information is available that would indicate whether or not these pests are limiting the spread of the wild species.

A survey of the authorities on *Rubus* spp. could be implemented to determine what “anecdotal” information exists about pest epidemics in native *Rubus*. When information is lacking, disease surveys could be conducted. Experiments could be conducted using a large number of genotypes of a single potentially weedy species. These plants could be used to screen for levels of resistance that might occur in native populations. Plants could be inoculated with a wide range of potential pathogens to determine if broad-spectrum resistance already exists. Due to the wide range of genetic variability within species and the distribution of native species, small-scale field trials to determine the extent of resistance in natural populations are less applicable than trials that test a wide range of genotypes.

**Table 2. Raspberry and blackberry pests.\***

<u>Rubus Pest</u>	<u>Status of Resistance</u>
Anthracnose ( <i>Elsinoe veneta</i> <sup>1</sup> )	Available through conventional breeding
Cane blight ( <i>Leptosphaeria coniothyrium</i> <sup>1</sup> )	Red raspberry- <i>R. pileatus</i> hybrids
Spur blight ( <i>Didymella applanata</i> <sup>1</sup> )	Conferred through “H gene.” <i>Rubus</i> spp.
Gray mold and Fruit rot ( <i>Botryotinia fuckeliana</i> <sup>1</sup> ) (anamorph: <i>Botrytis cinerea</i> )	Engineered
Orange rust ( <i>Arthuriomyces peckianus</i> and <i>Gymnoconia nitens</i> <sup>1</sup> )	Blackberry and red raspberry
Phytophthora root rot ( <i>Phytophthora</i> spp. <sup>1</sup> )	Cultivars of red raspberry
Bluestem ( <i>Verticillium</i> spp. <sup>1</sup> )	None
Raspberry bushy dwarf virus	Engineered

\*Ellis et al. 1991

<sup>1</sup> reported from wild relatives

## BLUEBERRY (*VACCINIUM*)

Four species of blueberries are cultivated: highbush (*V. corymbosum*), lowbush (*V. myrtilloides* and *V. angustifolium*), and rabbiteye (*V. ashei*) (Caruso and Ramsdell 1995). Highbush blueberries are the most commonly cultivated of the group, with approximately 100 cultivars; the most common is Bluecrop. More than 20 cultivars of rabbiteye have been developed, and although cultivars of lowbush blueberry have been developed, these are rarely planted. Highbush and rabbiteye blueberries are planted in rows, which may be in raised beds. Low vigor canes are removed annually from highbush types everywhere, and bushes are regularly hedged in the southeast. Lowbush blueberries are allowed to grow in natural stands. These are managed with mowing or burning to rejuvenate stands.

Weed management is extremely important in blueberry production; the plants are not strong competitors with most weeds. Pre-plant weed control is of utmost importance. In established fields, mulching, cultivation, and herbicide application are used in an integrated approach to weed management.

Feral blueberries are not found in agricultural fields and would be unlikely to become weeds due to the introduction of pest resistance traits. Highbush blueberries are very closely related to wild relatives, and lowbush blueberries are

undomesticated from a breeding standpoint. Pests occurring on blueberries in commercial areas occur on other native species (Table 3) (Farr *et al.* 1989). Blueberry viruses, such as shoestring and leaf mottle, have been documented in wild populations.

Introgression of pest resistance traits into wild *Vaccinium* is assumed to be due to their genetic similarity. Numerous hybrid swarms between cultivated and wild species exist in Michigan. However, it is highly unlikely that additional traits would lead to an increase in weed problems with this group.

In the case of all three of the berry groups discussed, it is unlikely that pest resistance genes that target a single pathogen or group of insects would cause significant increases in weed problems. There is concern, however, about broad-spectrum resistance genes. It was determined that a simple survey of authorities should be made to increase our knowledge base. A single question could be posed:

“What diseases, nematodes, or insect pests have you observed on native species of *Rubus*, *Fragariae*, or *Vaccinium*? Based on information from your observations (not lists of diseases in the literature), please indicate the relative abundance or impact of these pests on the native species.”

**Table 3. Blueberry pests.**

<u>Blueberry Pest</u>	<u>Status of Resistance</u>
Phytophthora root rot ( <i>Phytophthora cinnamomi</i> <sup>1</sup> )	Some (highbush and rabbiteye)
Botrytis blight ( <i>Botrytis cinerea</i> <sup>1</sup> )	None
Mummy berry ( <i>Monilinia vaccinii-corymbosi</i> <sup>1</sup> )	None
Stem blight ( <i>Botryosphaeria dothidea</i> <sup>1</sup> )	Limited (highbush)
Stem canker ( <i>Botryosphaeria corticis</i> <sup>1</sup> )	Limited (highbush)
Bacterial canker ( <i>Pseudomonas syringae</i> )	Highbush only
Blueberry scorch carlavirus <sup>1</sup>	Highbush only
Blueberry shock ilarvirus	
Blueberry shoestring sobemovirus <sup>1</sup>	Highbush
<i>Xiphenema americanum</i> , <i>Pratylenchus penetrans</i> , and <i>Meloidogyne carolinensis</i> <sup>1</sup>	Cultivars available

<sup>1</sup>pests known to occur on other *Vaccinium* spp.

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