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The Wisconsin Cranberry of the Future: A Basic Plant Breeding Approach

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For only about five years now, we have been pursuing a limited breeding program with the overall goal of genetically improving the cranberry for Wisconsin growers. This work resulted in large part from the initiative provided by the Gottschalk Family Endowment to UW-Madison. This endowment has not only provided the funding to support these efforts, but as importantly has built a stable support base so that a long-term effort like plant breeding could be reasonably pursued. This presentation will give an overview of this breeding effort and highlight some of the early findings and accomplishments. This effort is still at the early stages of development and refinement; thus there is ample opportunity for grower input into the goals and working relationships of the program. We hope you all will feel free to discuss your ideas with us.

Principles on which we are basing our breeding program.

For the fist year of the effort, we spent considerable time discussing ideas with growers and colleagues. From this initial discussion, it became clear that Wisconsin growers faced a number of problems that were not of general interest to other major growing areas in the U.S. In addition, there were a number of limitations on the scope of the program that had to be realized and incorporated. The result is a breeding program with the following characteristics and objectives:

- -Non-duplication of the efforts of other breeding programs
- -Focus on problems and potentials of the Wisconsin cranberry industry
- -Must operate under limited resources
  - -No State of Federal dollars would be available to support the effort
  - -No new personnel could be hired to work on the effort
  - -Unlike all other breeding programs, no cranberry research station would be available, thus the field work had to utilize grower sites exclusively
- -Both practical and basic biology objectives should be pursued:
  - -Major practical goals:
    - -High yield
    - -Manageable plant:
      - -In sand or peat production sites
      - -In central or northern production areas
    - -Early and intense fruit color under Wisconsin conditions

-Cranberry biology goals
- -What is the basis of high yield in Wisconsin?
- -Shorten the time needed to breed cranberry so that progress could be rapid and costs minimized
- -Techniques to select for the desired traits needed to be refined
- -When would evaluations made under greenhouse conditions be useful?
- -What field plot designs are be most cost effective?
- -What particular genetic and heritable traits do various of cranberry cultivars offer when used as parents?
- -Is there any value to increasing the ploidy level in cranberry?
- -How far could cranberry be inbred? Is outbreeding essential to maintain vigor?
- -How can transgenic cranberry plants be used as parents in a breeding program?

Some of these goals have received more emphasis than others; these are highlighted in this report.

Shortening the breeding cycle for cranberry.

Utilizing both basic horticultural techniques and some aspects of plant biotechnology, we have developed a methodology that has shortened considerably the time it takes to produce a generation of new cranberry hybrids. This approach is outlined below:

Controlled flowering of potted plants
(Growth retarding treatments)
(Programmed chilling)
⇓
Greenhouse pollinations
⇓
Harvest of unripe fruit
⇓
Excision of seed and germination in sterile culture
⇓
Seedling micropropagation
⇓
Production of transplants
⇓
Field and greenhouse screening

This methodology has allowed us to produce hybrids in 1 to 2 years less time than would normally be required if the natural seasonal cycle of the cranberry was allowed to proceed. Not only has this allowed us to conduct the breeding program more rapidly, but because each generation of seedlings takes less total time to generate, costs are reduced.

Selection and evaluation techniques

What traits might be able to be screened in a preliminary way under greenhouse conditions, thus avoiding the cost and time of field evaluations of useless seedlings? Obviously, traits like manageability cannot be effectively evaluated under anything but field conditions. However, we have found that a trait like early and intense coloration of fruit may be effectively screened under greenhouse conditions. By comparing the relative development of fruit coloration of seedlings along with standard cultivars, differences in the biological tendency to develop early and intense fruit color can be detected. The worst seedlings can then be eliminated before field trials, thus saving considerably on time and costs.

Field plots are both expensive and time consuming. The major expense is maintenance of the plots. In planting a field trial of 100's of seedlings, it is essential that each seedling be kept physically separate from all the others for the 3 to 4 year trial period. Since cranberry is a vine, such physical separation involves periodic pruning of the vines encroaching on adjacent plots. In our experience, this is the single most limiting factor in determining how many plants can be evaluated under field conditions. Even with the very able assistance of the participating growers who have provided not only the site but often helped in the maintenance, properly managing small field trials of cranberries is a major undertaking.

Large individual plots of a seedling are easier to maintain than smaller plots because of a lower proportion of edge. However, large plots take more time to plant and occupy more of the grower's field space. Thus there is a compromise between plot size, maintenance requirement, and total amount of field space required. Presently, our scheme is as follows:

Micropropagated transplants
(Preliminary screening in the greenhouse)
⇓
Discovery field plots
(2'x2' to 4'x4')
(no replicates)
⇓
Performance field plots
(10' x 20')
(replicated)
⇓
Acre-sized plantings

Currently, we have four major field evaluation sites, three in the Cranmoor area and one in the northern production area. Hundreds of seedlings are being evaluated in the discovery plots and a number of early selections have advanced to the performance plots.

Early hybridizations and seedling responses

Our first set of hybridizations utilized a number of parental lines and were designed to both work-out all the methodologies needed to conduct a breeding program with cranberry and test the usefulness of these selections as parents:

- -Desired character and parental line used:
  - -Manageable plant, dependability: 'Stevens'
  - -Fruit size, vigor: 'Pilgrim'
  - -Early, intense color: Boone's 'Ben Lear' selections
  - -Vigor: 'Norman LeMunyon'
  - -Keeping Quality: 'Bergman'

Hundreds of seedlings resulting from crosses using these parents have been evaluated in discovery plots. The first year's harvest (1995) showed some intriguing results. Parents that were selfed produced seedlings that were distributed rather tightly in measured characteristics such as fruit coloration (Figure 1); that is, selfed crosses of a parent with dark colored fruit produced only seedlings with dark colored fruit. Hybrids between a dark colored parent ('Ben Lear 3') and a moderate colored parent ('Pilgrim') produced seedlings that showed a continuous array of coloration that ranged from low color to color somewhat higher than the highly colored parent (Figure 2). A similar continuum was seen with fruit size (Figure 3), one major component of yield. This data confirms our initial prediction that both of these traits are determined by a relatively large number of genes.

From these very early hybridizations, a number of seedling selections have been planted in performance plots. These early selections show both superior coloration and high vigor. In addition, some plants (e.g. 'Ben Lear #3') when used as parents have resulted in a higher percentage of poor performing seedlings than other parental lines (e.g. 'Ben Lear #8'). Such poor parental lines will not be used heavily in future breeding.

The results of this first work are encouraging enough to continue the evaluation of the seedlings. In addition, the information we have gained has allowed us to decide how to conduct more hybridizations which will emphasize the best performing plant materials.

Your input.

We are at a decisive stage in this program. Utilizing the resources currently available to us, we are maxed-out; that is, we cannot expand the program any further without more money and labor input. We do feel that the program is large enough to be able to make some important progress in accomplishing the breeding goals, however no new goals can be included without either more input or eliminating some of the current activity. Thus a number of questions arise that you should consider:

- -Do you agree with the goals of this young breeding program?
- -Are there goals that you think are VERY important that need to be included?
- -Is the program OK at its present size or would you like to see it move along faster?

In addition, we would be very interested in hearing about any special selections that you might have in the "back 40" that may have traits that would be useful in such a breeding effort. If so, we may be able to include some of these in our evaluation plots.

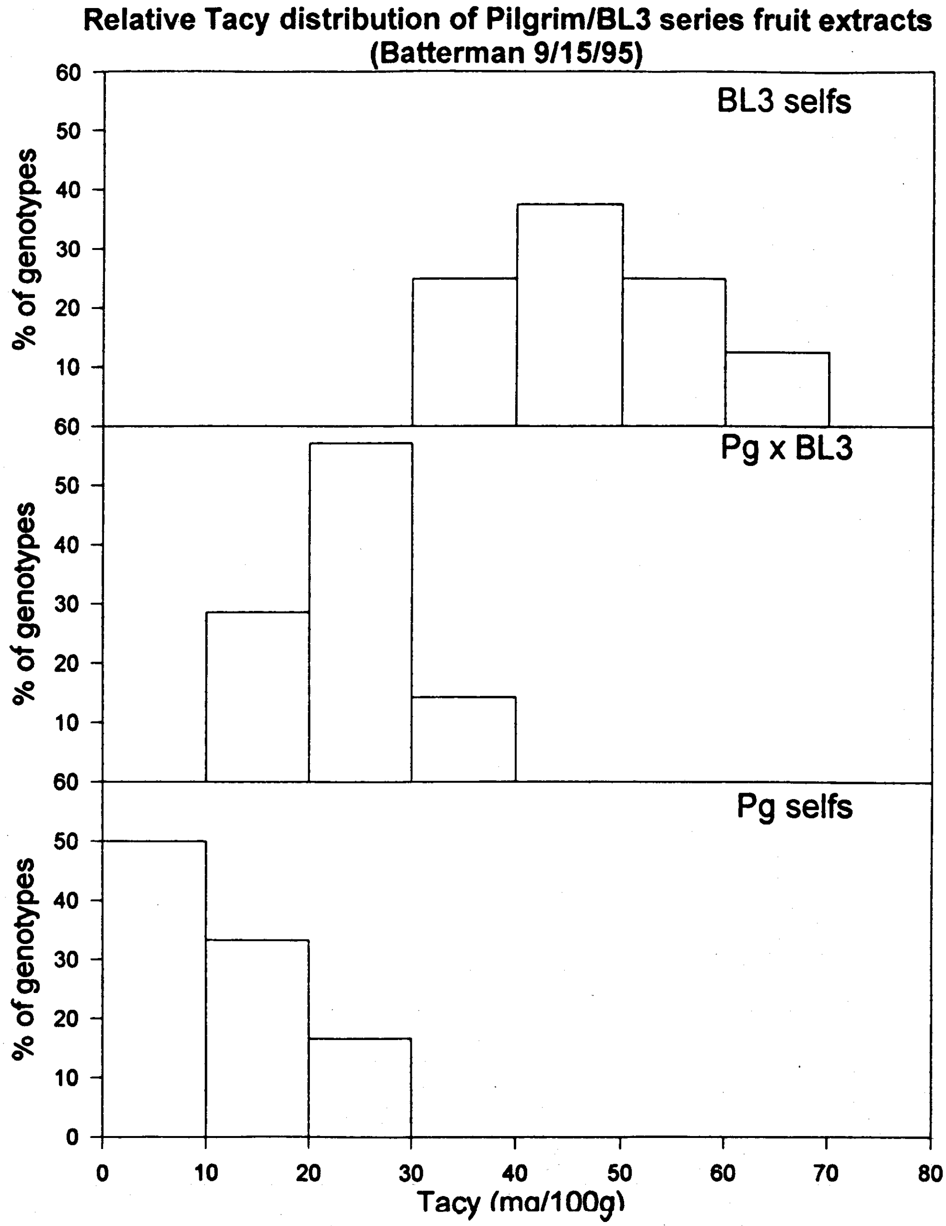

Figure 1. The fruit pigment concentrations of seedlings from three different crosses: top, seedlings from selfed 'Ben Lear #3'; middle, seedlings from a cross of 'Pilgrim' and 'Ben Lear #3'; bottom, seedlings from selfed 'Pilgrim'. Seedlings grown in field plots and harvested 9/15/95.

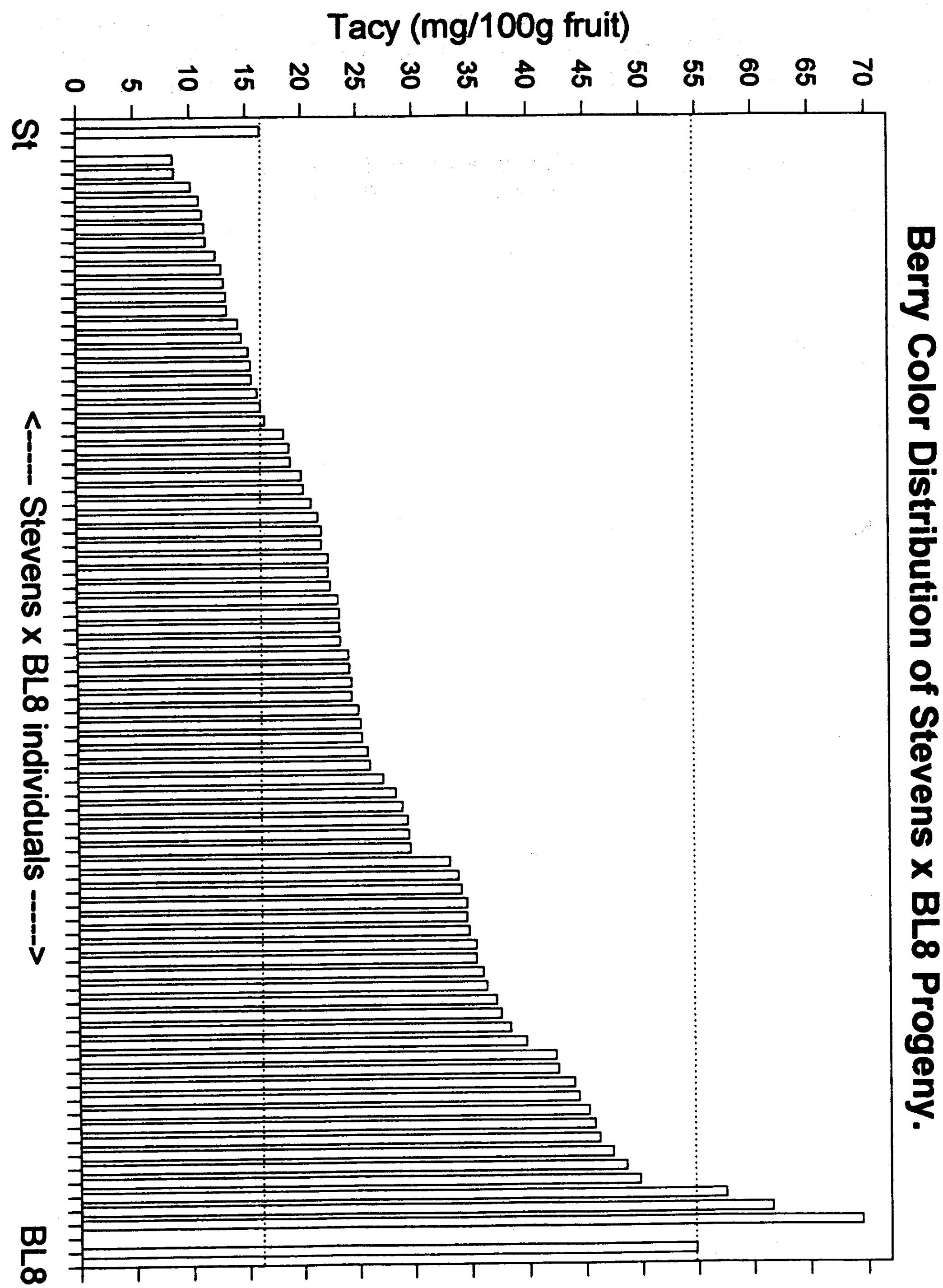

Figure 2. The distribution of berry color in seedlings and parents of a cross between 'Stevens' and 'Ben Lear #8'. Plants grown in the field and harvested in 1995.

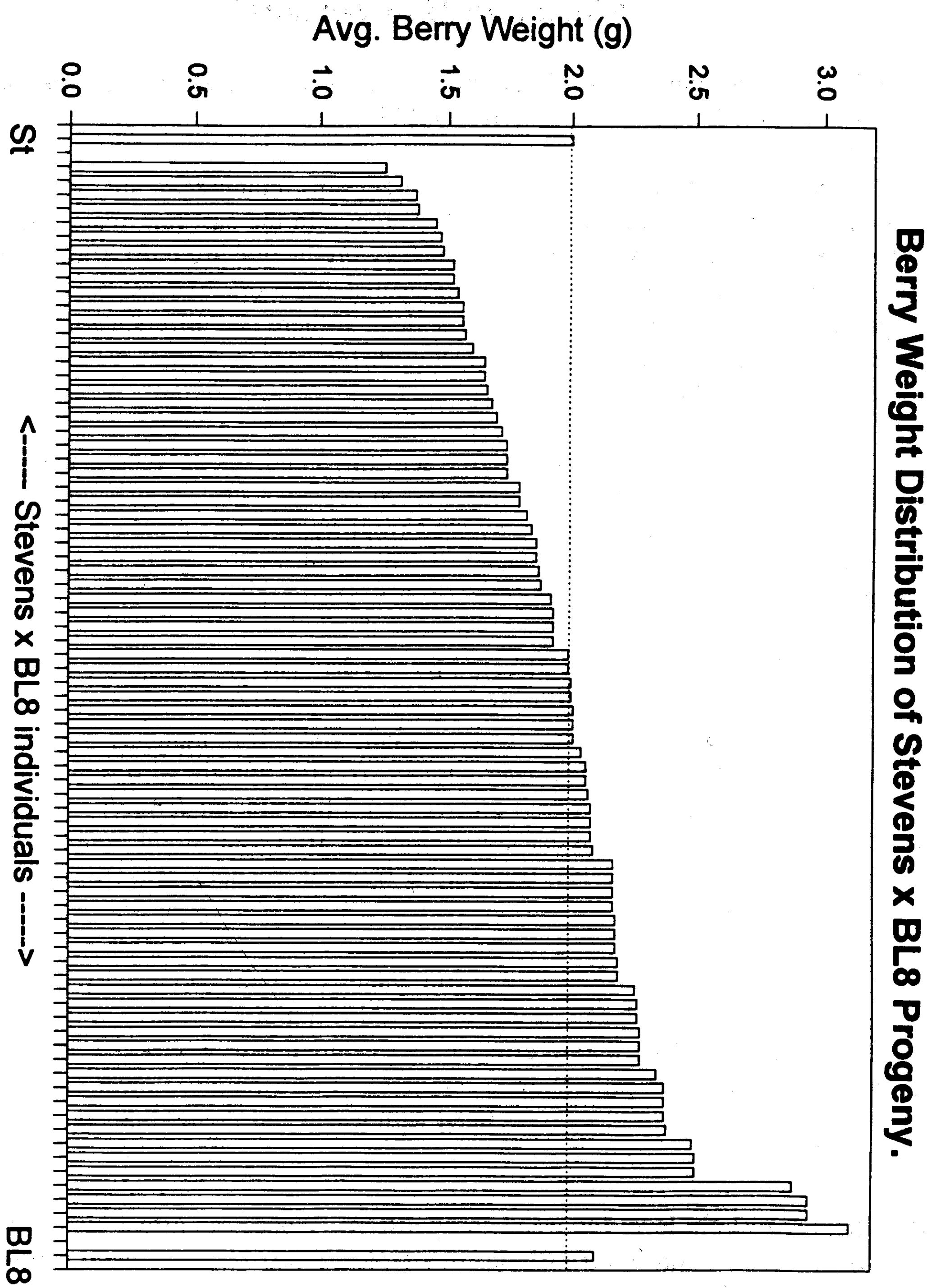

Figure 3. The distribution of berry size in seedlings and parents of a cross between 'Stevens' and 'Ben Lear #8'. Plants grown in the field and harvested in 1995.

Weed Competition Effects on Cranberry: When and How Serious?

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Based on numerous surveys of agricultural crops, cranberries are more prone to yield losses due to weeds than almost all other crops. In the past few years I have been trying to document which parameters of cranberry production are most affected by weeds, the threshold levels of weed populations that affect cranberries, how one year's weed populations affect the following year's yield, if there is a difference in variety response to weed competition, and the effect of differences in time of weed competition on yield and fruit quality.

This research was conducted by first selecting hundreds of weed patches within numerous cranberry beds over three years. Weed densities at these sites were measured based on how much light the weeds prevented from reaching the cranberry canopy. I then measured dozens of yield component variables and tried to relate them back to the weed density.

Vine and yield parameters most affected by weeds: The yield component parameters fell out into three categories in terms of severity of impact by weeds. *Severely influenced*: total cranberry biomass (fruit + vine), yield, fruit number, fruiting uprights, total vine fresh weight, flower number, fruiting upright with flower buds (return bloom), fruiting uprights with greater than 3 flowers; *moderately influenced*: flower bud density, total upright density, fruit color, fruit size; *slight influenced*: non-fruiting uprights with flower buds, fruit set, % fruit rot, runners, non-fruiting uprights, flowers per fruiting upright.

From this data it was evident that fruit yield is influenced more by weeds than fruit quality parameters such as size and color. Also important to note is that this data suggest which parameters are most affected by shading due to weeds and thus, most sensitive to deficiencies in carbohydrate resources. For example, flower bud formation on fruiting uprights (return bloom) declined with weeds but flower buds on non-fruiting uprights did not.

The critical threshold level for weed competition: Weeds can affect cranberries in a linear fashion or in a non-linear fashion. In a typical linear response there is no critical threshold; with each incremental increase in weed density, there is a corresponding decrease in yield. In a non-linear response the decline in yield does not occur until a certain density of weeds is achieved. Figures 1 and 2 show typical response curves for yield and fruit size for different weed species and varieties. In general, yield and fruit quality respond linearly. Density of fruiting uprights and fruiting uprights with return bloom, however, respond non-linearly. The threshold value for these later parameters appears to occur when weeds block greater than 50% of the sunlight reaching the cranberry vines.

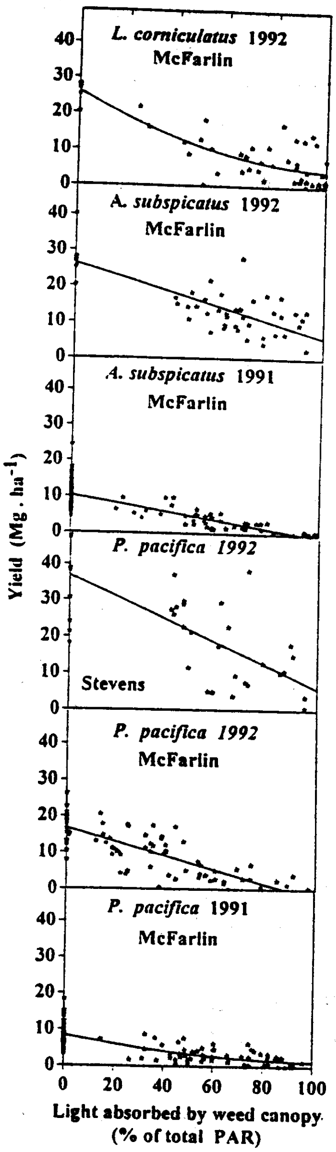

Figure 1.

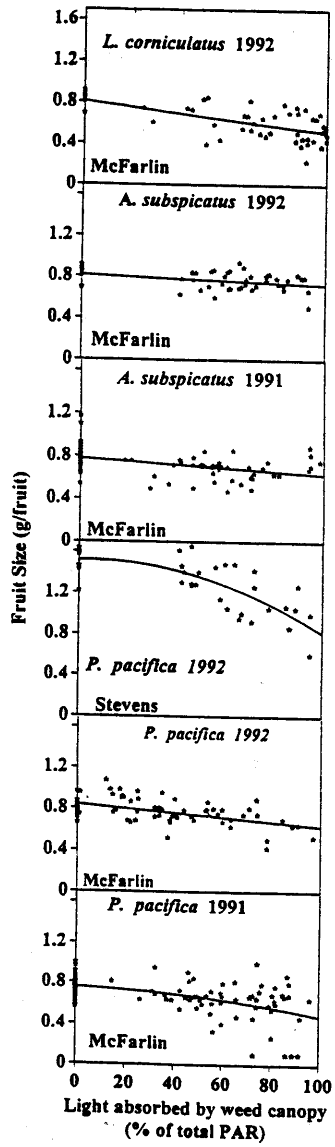

Figure 2.

Figures 1 and 2. Relationship between cranberry yield (Figure 1) and cranberry fruit size (Figure 2), and the percentage of total sunlight absorbed by various weed canopies (total weed coverage =100% light absorbed). To convert yield to bbl/ac multiply by 10. The weeds represented are Lotus corniculatus (Birdsfoot Trefoil), Aster subspicatus (Purple Aster), and Potentilla pacifica (Pacific silverleaf). The steeper the slope of the line, the greater the impact of weeds on the parameter.

Long term effects of weeds: The above data indicate that the current season's carbohydrate accumulation necessary for fruit production is limited by weed competition. Equally important, but less obvious, is that weeds have a severe impact on the fruit-carrying capacity of the crop for the following year. In fact, for Stevens we found that shading caused by weeds was more responsible for crop loss in the year following the shading than in the year of shading (Figure 3). The previous season's influence is likely a combined effect on reduced stored carbohydrate reserves, plant biomass available for photosynthesis in the following year, or flower bud formation.

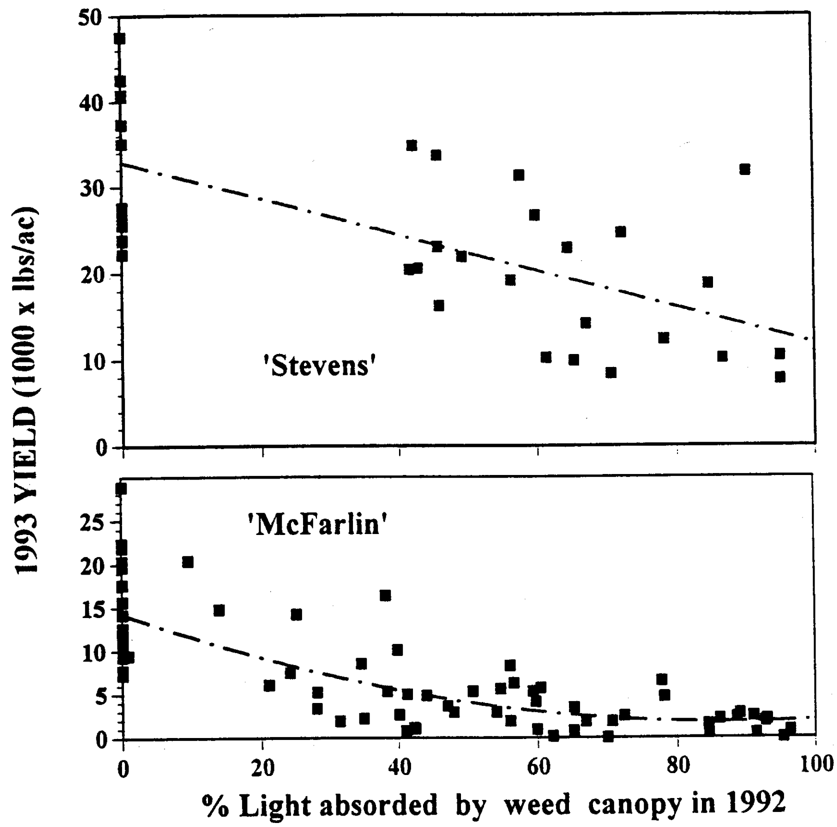

Figure 3. The effect of shading by weeds (% light absorbed by weed canopy) in 1992 on the yield of Stevens and McFarlin in 1993. Data imply that weed competition in the current year has a very significant effect on next year's yield.

Flower bud formation on fruiting uprights (return bloom) appeared to be much more sensitive to weed competition than flower bud formation in non-fruiting uprights. This suggests that biennial bearing in cranberry is resource limited and highly influenced by weed populations. To evaluate that concept, I computed the relationship between weed populations in 1992 and the density of fruiting uprights in 1993. I found that fruiting upright density significantly decreased as weed populations in the previous year increased (Figure 4).

Varietal response to weeds: Do all cranberry beds respond to weeds in the same fashion? Should we be equally concerned about weeds in a moderately productive bed as in a highly productive bed? Two interesting conclusions were reached in this regard. First, the lower yielding McFarlin variety was less sensitive to shading than the higher yielding Stevens variety. Stevens yield, for example, declined at a 3-fold greater rate for a given weed population than McFarlin. Stevens were also quite sensitive to weed populations in the previous season while McFarlins were much less affected. Fruit size on Stevens was also more significantly reduced by weeds than on McFarlin. Second, within any one variety the higher the yield potential of a bed the greater the percentage loss caused by an equivalent number of weeds. That is, it pays to have fastidious weed control on high-producing beds but not necessarily on low-producing beds.

Critical period of weed infestation and type of weed responses: Cranberries may be especially sensitive to interference from weeds during a specific phenologic period. Shading by weeds during flowering, for example, could restrict bee visitation and reduce fruit set, or shading after flowering could restrict carbohydrates required for fruit set. Our data in this area is more sketchy. Artificial shading experiments by Dr. Roper in Wisconsin indicated that shading during the post-bloom period had a more severe impact on fruit set than shading during the pre-bloom or pre-harvest interval.

How and when a weed causes shading is a function of weed canopy architecture. Weeds belonging to the Rush family, for example, have a very non-imposing leaf structure and are not likely to restrict bee visitation. Some weeds, however, some may be so dense at the time of cranberry bloom as to restrict pollination by bees. Other weeds, such as Potentilla or Birdsfoot Trefoil can partially or completely occlude light from a cranberry canopy in June depending on weed vigor. In fact, heavy Trefoil infestation can become so thick as to cause beds to become quickly void of vines. Other weeds such as aster do not reach full canopy development until August and, therefore, have less early season shading impact. Aster also has a single erect stem that allows more light penetration when the sun is at oblique angles than weeds with a dense canopy. More important to how and when a weed may shade the vines is how aggressive and difficult to control is the weed. That is, a weed may not provide much initial shading during the critical time periods but if it eventually takes over the bog and cannot be easily controlled, it is a disaster.

Conclusion: Weeds are one of the most critical limiting factors of cranberry production. Their influence extends across many yield components and across multiple years. Their effect on production often occurs at very subtle levels of weed densities. A special emphasis should be placed on weed control in cranberry beds with high yield potential.

Herbicide Physiology: Why do I see what I see?

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Herbicides are an important tool in the production of cranberries. Production statistics show steady increases as herbicides became more available and widely used. Weeds compete with cranberry vines for light, water and mineral nutrients. Left uncontrolled weeds will significantly reduce yields and fruit quality.

Herbicides used on cranberries can be grouped in a variety of ways. For the purposes of this article I'm going to group them as pre-emergence and post-emergence. Simply put, this is a grouping as active against germinating seeds and seedlings or active against mature weeds. Within each group of herbicides the different commercial products control a different range of weeds. Not all pre-emergence herbicides will control all emerging weeds. You need to compare the weed spectrum of the available products and compare that list to the weeds commonly encountered on your marsh.

Herbicides and plants

The reaction of plants, both weeds and crop plants, to an herbicide application is determined by both the plant and the soil and the interaction between the two and with the environment (weather). Plant response to an herbicide is difficult to predict exactly because so many variables are involved. These factors may also interact with each other to create even more complexity. Plant responses to herbicides depend on:

1. The uptake and absorption of the material
2. Translocation or movement within the plant
3. Ultimate fate of the herbicide within the plant
4. Effect of the herbicide on plant metabolism

In order to be effective, an herbicide must enter the target plant. Different herbicides gain entry into plants in different ways and different plants are susceptible to herbicide entry at different locations. Herbicides can enter plants through leaves, roots, shoots and stems.

Post emergent herbicides generally are absorbed through the leaves. Leaves are covered with a waxy cuticle layer that prevents water from escaping and, therefore, also prevents aqueous solutions from entering the leaf. The cuticle tends to be thinner at trichome bases and that is a preferential site of entry. Also, different plants have cuticles made of different types of waxes, some of which are more penetrable than others. Surfactants also help penetration by allowing greater contact between the herbicide solution and the leaf. However, surfactants may also alter the selectivity of an herbicide if the selectivity depends on differences in leaf absorption.

Pre-emergent herbicides or soil active herbicides usually are absorbed by plant roots before they are active. However, some work through vapor action near the soil surface. The primary root region where herbicides are absorbed is 5 to 50 mm behind the root tip. In this area the xylem is functional, but the casparian strip that stops movement of materials into the roots is not completely lignified and therefore is not a significant barrier. Herbicides may be taken up passively by roots while others require the plant to expend energy to take them up. For the most part herbicides enter plant roots passively.

Once an herbicide enters the plant it may move to a location other than where it was absorbed to be effective. Two general pathways exist for compounds to move within plants. These are the xylem (apoplast) and the phloem (symplast). Movement in the xylem is passive and follows the same pathway as water. They enter the xylem and move along with the water to leaves and fruit. The driving force is water evaporating from leaves through transpiration. Movement in the phloem is generally from areas of high sugar concentrations (mature leaves) to areas where sugar is being utilized (developing fruit, growing points, young leaves). If herbicides that are to move through the phloem are applied to too high of concentrations, they kill or disrupt phloem movement and cannot be transported throughout the plant for a "complete kill".

Most plants have no way to rid themselves of materials once they have entered the plant. In order to protect themselves plants must either sequester the foreign materials or metabolize them into something less harmful by adding molecules onto them or splitting them into smaller pieces that may be less harmful. When the phytotoxicity of herbicides is reduced we call it inactivation; when it is enhanced it is called activation. This can be the basis of selectivity as well. If a plant can metabolize or sequester a chemical it won't be toxic. On the other hand, if a plant will metabolize a relatively benign substance the result could be toxic to the plant.

Ultimately to be effective, an herbicide must disrupt some essential plant function in such a way that the plant dies. This is called the "mode of action". One needs only to think of all of the processes that take place in plants to devise approaches to kill plants. Herbicides may interfere with a single process or multiple processes within a plant. Weed resistance is more likely to develop if only a single process is interrupted, but the opportunity for selectivity is greatest then. Often, the exact mode of action of herbicides is not known. A good example is 2,4-D. This herbicide has been widely used for 50 years and yet the exact mode of action is not known.

Selectivity to herbicides is not entirely dependent on herbicide chemistry. The plant also plays a significant role. Young plants generally have a thinner cuticle than older plants so more herbicide can enter young plants. Young plants also have more actively growing tissues, thus are more susceptible to growth interruption than older plants. In general, fast growing plants are more susceptible to injury than slow growing (mature) plants. Also, seedlings making the transition from stored reserves to surviving on their own are very susceptible.

Herbicides and soils

Soils also play an important role in determining herbicide activity and longevity in the soil. Important soil properties include soil texture, temperature, moisture content, oxygen content, soil micro-flora and fauna, and exchange capacity.

The persistence of an herbicide in the soil is important. Too short of persistence and weeds come back quickly, too long of persistence precludes planting susceptible crops the following year. For cranberry production, persistence is desirable to provide long term weed control. Herbicides are broken down in the soil by these processes:

- **Degradation processes:**
  a) Biological decomposition
  b) Chemical decomposition
  c) Photodecomposition

- **Transfer processes:**
  a) Adsorption onto soil particles
  b) Leaching through soil
  c) Volatility
  d) Surface runoff
  e) Removal in harvested plants

Herbicides can be degraded by soil microorganisms or by the metabolic systems of higher plants. When an organic (carbon containing) herbicide is applied to the soil, some microorganisms can use it as a food source. These microbes then multiply and hasten the degradation of the herbicide. Once the herbicide is gone they generally decrease in number. Optimal environments for microbes are warm, moist and have an adequate supply of oxygen and mineral nutrients.

Chemical decomposition is the process of decomposition through chemical means without any organism being involved. Some herbicides break down in the presence of water. Others are sensitive to low or high soil pH.

Photodecomposition is the degradation of herbicides by light. Most herbicides absorb ultraviolet light. The absorption of light energy may allow bonds to be broken or new bonds to be made. This may activate or deactivate a given molecule.

Besides decomposition, herbicide activity may be lost as the active ingredient is moved out of the rooting zone. This can happen in a number of different ways. Herbicide can be lost with soil as soil erodes. Herbicide can leach through the soil as water from rainfall or irrigation moves downwards or laterally. This depends on the nature of the soil (sand > silt > clay > organic matter), the water solubility of the herbicide and the amount of water put through the system. All chemicals have a vapor pressure and a certain amount of material will be lost through volatilization into the air. Herbicides can be lost along with surface runoff water. This can be a big problem if runoff ends up in non-target areas. Herbicide that has been absorbed by plants will be removed from the field in the harvested product. This may not be a significant issue for cranberries since such a small portion of the biomass is harvested.

Herbicide application

Application technology is the final variable to cover in this article. Regardless of the biological activity of an herbicide or the susceptibility of a weed or the tolerance/resistance of the crop, weed control will be inadequate if the herbicide is not applied correctly. Application factors to consider include:

- Calibration
- Even application
- Timing

In order to be effective, the correct amount of material must be applied to a given land area. This amount is always specified on the product label. Poor calibration of application equipment is a primary reason for poor weed control. Calibrate your equipment often to assure the correct amount of product is being applied.

Not only must the correct amount of material be applied, it must also be applied evenly over the surface area (soil or leaf) to be effective. This is a consideration for both ground and air applications. Overlap and misses are of constant concern with air application (along with off target application). Cantilevered booms are inherently unstable and instability increases with boom length. While moving along a dike the boom arm may move up and down and forwards and backwards resulting in uneven application. For liquids, if the nozzles are not evenly spaced or if they are spaced too widely striping of the field will occur.

Timing of application is the last area of concern. If pre-emergent herbicides are applied after weeds have germinated and begun to grow poor control will result. Timing of post-emergent herbicide applications is even more important. For crop safety, 2,4-D granular applications can only be made in the spring before growth begins. On the other hand, Roundup (glyphosate) will provide best control when application is made later in the season when more nutrients are translocated to the roots.

Herbicides are an important component of cranberry cultivation. Using these tools wisely will allow their use for a longer period of time and will provide better weed management. Most problems of non-control are related to improper use of a product.

Table 1. A comparison of pre-emergence herbicides labeled for Cranberry in Wisconsin.

Herbicide Trade (common)	Solubility in water	Acute oral toxicity	Acute dermal toxicity	Weeds controlled	Degradation	Mode of action
Evital, (norflurazon)	low, 28 ppm @25°C Not easily leached, adsorbed by clay & OM	8 g/kg	20 g/kg	Annual grasses and broadleaves	In soil, degraded by soil microorganisms. Also volatilization & photodecomposition. Half life[z] is 45 to 140 days depending on soil conditions.	Inhibits biosynthesis of carotenoid pigments that protect chlorophyll. Susceptible seedlings emerge with yellow leaves and die. Cranberry is not susceptible because it can metabolize Evital.
Devrinnol, (napropamide)	73 ppm @ 20°C Resists leaching	>5g/kg	>4.6 g/kg	Most annual grasses & many broadleaf weeds	Slowly decomposed in soil by microbes. Half life in loam soil of 8 to 12 weeks.	Exact mode of action not known. Inhibits development and growth of roots, particularly grass roots. Metabolized in mature plants.
Casoron, (dichlobenil)	18-25 ppm @ 20°C Attaches tightly to OM, won't leach	2.4 g/kg	1.3 g/kg	Many broadleaf, grass and sedge weeds.	Slowly decomposed in soil by microbes. Half life in soil of 2 to 12 months	Inhibits growth of rapidly dividing tissues such as shoot or root tips. Inhibits phloem transport. Translocates quickly in xylem from roots to leaves & tips. Also absorbed in vapor phase by leaves.
Princep, (Simazine) Triazine	5 ppm @ 20°C Adsorbs to clay and OM in soil. May leach, little lateral movement	>5.0 g/kg	>3.1 g/kg	Emerging annual grass & broadleaf weeds.	Persistent and active in soil for extended periods. Sometimes > 1 year	Inhibits photosynthesis by interfering with electron transport.

z. Half life is defined as the length of time it takes for half of the material originally present to be gone.

Table 2. A comparison of post-emergent herbicides labeled for cranberry in Wisconsin.

Herbicide & class Trade (common)	Solubility in water	Acute oral toxicity	Acute dermal toxicity	Weeds controlled	Soil Degradation	Mode of action
Roundup, (glyphosate)	1.2% @25°C	4.3 g/kg	7.9 g/kg	Any green tissue. Selectivity is by selective application	Degraded by microbes in the soil, nonpersistent.	Inhibits the formation of some essential amino acids.
Touchdown (sulfosate) Non-bearing only	miscible	0.78 g/kg	>2 g/kg	Any green tissue. Selectivity is by selective application.	Degraded by microbes in the soil, nonpersistent.	Inhibits the formation of some essential amino acids.
Poast, (sethoxydim)	48 ppm @25°C	2.7 to 3.1 g/kg	Little or no reaction	Actively growing grasses	Persistent in soil only 4 to 5 days	Inhibits lipid synthesis by blocking acetyl-CoA carboxylase. As a result treated plants can't repair or create new membranes.
Fusilade, (Fluazifop-P-butyl)	2 ppm @25°C	3.3 g/kg	>2 g/kg	Actively growing grasses	Some persistence in soil. Half life [z] of about 3 weeks.	Inhibits lipid synthesis by blocking acetyl-CoA carboxylase. As a result treated plants can't repair or create new membranes.
Weedar 64 Weed Rhap 20G (2,4-D)	900 ppm @25°C	0.3 to 1.0 g/kg	—	Broadleaf weeds	Degraded by soil microbes. Adsorbed onto clay and OM in soils. Persists in soil for 1 to 4 weeks.	Exact mechanisms unknown. At low concentrations growth and abnormal RNA produced. At high concentrations growth and RNA production reduced.

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The Wisconsin Cranberry of the Future: A Biotechnological Approach

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For the past 6 years, the Wisconsin Cranberry Board has supported a project to determine how modern plant biotechnology (genetic engineering) might be used to genetically improve cranberry selections. Recently, Ocean Spray has also helped to support the project. The project has evolved over this period to emphasize various goals; some of these have been completed while others are continuing:

- -Micropropagation and transplant technologies useful in establishing new plantings
- -Incorporating resistance to the pest Blackheaded Fireworm into leading cultivars by genetically engineering cranberry with *BT* genes.
- -Incorporating tolerance to the biorational herbicide, Bialophos, into leading cultivars by genetically engineering cranberry with the *BAR* gene.

Since detailed reports on all of this work have been written annually to the Wisconsin Cranberry Board, Inc. and to Ocean Spray and have been published in the annual compendium of such reports, the work will not be detailed here. Instead, some of the highlights will be noted and some of the implications of the work will be discussed.

This project is not unlike many others working with other crops in that the goals and the genes utilized to achieve these goals are common. However, the project is very much unlike other similar projects in that the resources used to accomplish this work are comparatively much less. The work has only been feasible to approach because of the cooperation of a large number of individuals and organizations ranging form growers, to students, to companies, to government agencies.

Working relationships

The project has continued to act as a bridge between the cranberry industry and biotechnology laboratories and companies. This has resulted in agreements to provide access to proprietary technologies that may be useful to genetically improve cranberry. Examples of these technologies include the following:

- -Use of the 'Accell' gene gun, a proprietary system for transferring genes to cranberry (Agracetus Company)
- -Use of gene constructs containing genes for insect resistance (*BT*) and herbicide tolerance (*BAR*) and genes essential in recovering transformed plants (*GUS*, *KAN*) (Agracetus company)
- -Access to a vast array of *B.t.* strains for testing to determine the most useful strain for controlling pests of cranberry (Mycogen, Inc.)

Without access to such technologies, this project would not have been possible to carry-out. New working relationships are constantly being sought as new technologies advance to the commercial level.

In addition to working with scientific organizations, the project has coordinated with governmental organizations (State and Federal) to gain clearance to conduct the work. In particular, we have addressed all the environmental and other concerns that have been raised to date and now are able to conduct this work in open field situations.

Technique development

The project has perfected and proven a methodology that allows the insertion of foreign genes into cranberry and the recovery of genetically-engineered plants. This process has been patented and to our knowledge, this is still the only reliable method of genetically engineering cranberry. The method has a number of steps:

Sterile culture (microculture) of cranberry to provide large numbers of uniform stem pieces.

⇓

Induction of these stem pieces to produce miniature buds.

⇓

Gene insertion into the buds by particle bombardment.

⇓

Selection for further growth of only those cell/tissues/plants that have been genetically engineered.

⇓

Recovery of transformed shoots.

⇓

Micropropagation of these shoots.

⇓

Testing for gene presence and function.

In addition to the genetic engineering methods, numerous techniques have been developed to test the recovered cranberry plants for traits of commercial value. Such testing techniques include:

-Pest resistance:
  - -A method to culture Blackheaded Fireworm year-around in the laboratory.
  - -A rapid laboratory assay to screen for resistance of plants to blackheaded fireworm.
  - -Procedures to grow and test cranberry in areas outside the normal production regions in Wisconsin.

-Herbicide tolerance:
  - -A laboratory and greenhouse assay for tolerance to Bialophos.

Status of the work.

Compared to efforts on other crops, this is a very small program for a genetic engineering project. Nevertheless, some very important milestones have been achieved:

-Cranberry can now be routinely genetically-engineered.
-Inserted genes function and are stable in the plant even in the field and through multiple growing seasons.
-The inserted genes are inherited between sexual generations, thus these transformed plants can be used as parents to pass on the new traits.

Pest resistance.

In regards to resistance to Blackheaded fireworm, plants have been obtained that show more resistance than the current commonly grown cultivars of cranberry. For example, in the field test plots this year, plots of transformed lines of 'Pilgrim' and 'Stevens' had 30 to 40% less Blackheaded fireworm mature successfully when feeding exclusively on them than on the regular 'Pilgrim' or 'Stevens' foliage. Although these results show that the insertion of the *BT* genes do give resistance to this pest, the resistance obtained is not at the level (100% kill) that has been observed in other crops. Our most likely explanation for the reduced effectiveness of this *BT* gene in cranberry is that cranberry contains chemical compounds that interfere with the action of *BT*. We have direct evidence to show this effect. If we want to increase the pest resistance conferred by inserted *BT* genes, we will probably have to repeat the work but use a newer, more powerful *BT* gene so that the inhibitory compounds are overwhelmed by very high levels of the *B.t.* pest toxin produced in the plant.

However, there is an open question as to just how much *B.t.*-based pest resistance we want to engineer into cranberry. The problem revolves around the need to avoid a situation where the planting of genetically-engineered *B.t.* cranberries results in the emergence of Blackheaded Fireworm populations that are resistant to *B.t.* There are two schools of thought on this issue. A high dose strategy approach says that a cranberry plant with a very high level of *B.t.* toxin will eliminate all Blackheaded Fireworm so that none will be surviving to breed. However, if eventually some pests do mutate and are capable of surviving such high doses of *B.t.*, then the use of *B.t.* in any form as a pest control option will be eliminated.

A second strategy, the 'low dose' approach, uses *B.t.* in conjunction with other control (e.g. natural predators, insect diseases, traps) on the insect population so that it is not just the *B.t.* that is impacting the pests. In this situation, since *B.t.* is not the only stress on the pests, the insect population would not likely evolve a resistance to *B.t.* However, this approach is more complex and thus more difficult to effectively implement and manage.

It would also be interesting to know if under field conditions, the low level of *B.t.* in the plant would make spray applications of *B.t.* more effective. Thus a grower would be able to control Blackheaded Fireworm by combining genetically-engineered plants with biological pesticides. Different forms of the *B.t.* could be used in the spray than what was engineered into the plant, thus providing a complex spectrum of controls and minimizing the development of pest tolerances.

What we may have to do is to test just how effective our current transformed cranberry lines are in actual cranberry field production situations. This will require the planting of genetically-engineered cranberry in production areas (not now approved) and on sites where no or few chemical controls for pests are utilized. The later is important since any reduction of beneficial insects (predators) by agrochemicals would defeat the low dose strategy. Unfortunately, this test may have to await an effective, non-agrochemical control for tipworm.

Herbicide tolerance.

Our original approach to recovering cranberry plants that contain the inserted *BAR* gene giving tolerance to the herbicide was not successful. We used the herbicide itself to select for those cells that contained and were expressing the *BAR* gene, however we soon discovered that the non-transformed cranberry tissues were able to develop a tolerance to the herbicide. Thus the herbicide itself was not an effective way to discriminate between genetically-engineered plants and regular plants, all growing in the same test tube. We are now using a new gene construct which will allow us to use the same recovery procedures that have proven so effective with the *BT* work.

Future decisions.

If the work proceeds as expected, within a few years we will have genetically-engineered plants that will need to be tested in cranberry production areas in Wisconsin. The initial restrictions on these tests are liable to be rather severe. For example, we may be required to limit as much as possible the escape from the plot of any plant material that could survive outside the plot boundaries. In addition, we may have to destroy the plot after the test and verify that all genetically-engineered plant material is dead or removed. These restrictions are certainly not insurmountable, however the test will require an area that (1) is relatively isolated from other cranberry production areas and (2) a grower is willing to maintain but will not be able to harvest for the life of the test.

Any takers?

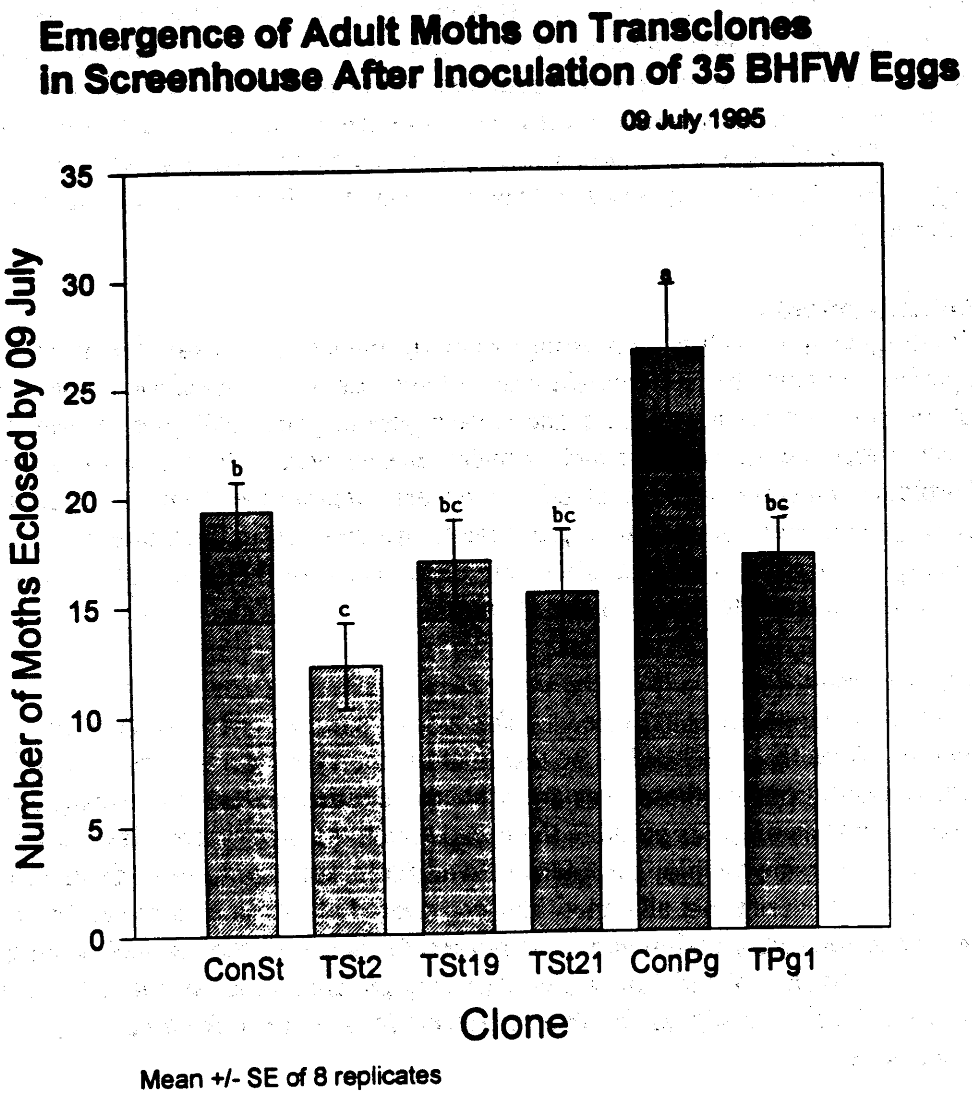

Figure 1 . Emergence of adult BHFW moths on transclones and control cultivars in the screenhouse approximately one month after inoculation with 35 BHFW eggs. Bar indicates the mean ± S.E. of 8 replicates.

Weed Management, Herbicides and Soil pH

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Good weed management has to be one of the most challenging aspects of a cranberry grower's task. There are only a few registered herbicides, it is doubtful more will ever be registered, the rates required for weed control either damage the cranberries or are not legal, it is not possible to till, and the overall soil/water environment is not user friendly for herbicides. Therefore, a grower has to fine tune and understand the system from both an herbicide and non-herbicide approach in order to succeed. This paper addresses those concerns in three points: 1) the interaction of the soil environment (microbiology, chemistry and moisture) with herbicides, 2) the interaction between weed biology and soil pH and, 3) the fine tuning of herbicide application timings, rates and combinations.

1) Herbicide and soil environment interactions.

Microbiology: Unfortunately, weed control varies tremendously among sites and across years. Several years ago we began to notice a loss of efficacy in weed control after the second year of Devrinol use (Figure 1). By the third and fourth years, we were getting no weed control. This loss of weed control effectiveness with each successive use has been reported in the literature for Devrinol (Walker and Welch, 1992) and is termed, enhanced microbial breakdown. Basically, this means that the population of microbes that breakdown the herbicide increase with continued use. Therefore, the half-life in the soil continually decreases. The best solution to avoid this problem is to stop using an herbicide for several years. We are now trying to determine if other herbicides have this the same characteristic. Literature indicates that it happens with 2,4-D and our preliminary data suggest that it happens with Casoron.

Soil chemistry: Herbicide behavior in soil tends to follow the fundamental laws of physical chemistry. All herbicides have a binding affinity to organic carbon. The higher the binding affinity, the greater the propensity to be tied up in the soil matrix.

For example, we generated standard curves for Casoron in different soils commonly used for cranberry growing. Figure 2 shows that relationship on a logarithmic scale. It takes 2 ppm of Casoron to achieve 50% kill in sand and over 30 ppm of Casoron on peat. That is, weed control on peat requires ten-fold more Casoron than on sand. A knowledge of the organic matter content in your cranberry bed is vital for obtaining a balance between weed control and phytotoxicity. On soils with little organic matter, the herbicide half-life is also greatly diminished. Casoron may last for several years on a peat soil and only months on a sand soil. Based on these principles, we have found that multiple applications of Casoron on sandier soils are frequently better than a single application.

Soil Moisture. Without good drainage in a cranberry bed, herbicide response becomes unpredictable. Most growers are familiar with Evital puddling in the low spots. Other problems include soil moisture effects on herbicide persistence. Casoron persistence is shorter under unsaturated soil conditions (25 - 50% moisture) than saturated conditions (75 - 100% moisture) (Figure 3). This factor helps explain the higher frequency of Casoron damage on cranberry beds where drainage is poor.

2) Manipulation of the weed biology through altered soil pH.

Soil pH and weed biology: Soil pH greatly affects the composition of plant species occupying a given ecosystem. Many weed species occur within a limited soil pH range. A differential response to changes in soil pH between cranberry and a specific troublesome weed could result in a competitive advantage for the cranberry. Surveys in Massachusetts found that cranberry bogs with the greatest production and fewest weeds had the lowest soil pH. Soil acidification, therefore, may be a viable weed management practice in a cranberry planting for several weed species. For example, in leguminous weed species such as clover which are sensitive to soil acidity, modification in soil pH can be used to help provide control.

We have been conducting research on the use of sulfur for soil acidification of established cranberry bogs to determine efficacy of weed control and phytotoxicity, and the interaction of soil pH and herbicides.

The legume weed, Birdsfoot Trefoil is particularly difficult for West Coast growers to control. Figure 1 shows the change in Trefoil coverage with the change in soil acidification over several years. In the first year of treatment, Trefoil was not observed below a soil pH of 4.0; by the second year of treatment it was not observed below pH values of 4.5. That means that a lower pH is needed for quick control. Gradual control is accomplished at a slightly higher pH. In the same vein, control of some species by using soil acidification may actually take years. For example, it took three years to eliminated Potentilla with sulfur. Weed control by acidification appears to occur as the result of mineral imbalances and, therefore, takes time to express.

The acidification of most cranberry soils with sulfur is not permanent. The sand layers on which the cranberries grow were poorly buffered. Use of high pH water, for example, will cause the soil pH to drift back to its initial levels within a year. In cases of severe weed infestation, all weed control benefits will be lost unless additional sulfur is applied (see Figure 1). The amount of supplemental sulfur required for pH maintenance is usually less than what is needed for the initial pH reduction. This is usually because populations of autotrophic sulfur oxidizers are stimulated by sulfur applications. The need for maintenance doses should be determined by running soil pH values once or twice a year.

We learned the hard way that sulfur applied at too high a rate in a single application can be quite phytotoxic. Rates below 500 lbs./ac/application were usually safe; rates above 1000 were not. Therefore, several applications at 100 to 200 lbs/ac applied 4 to 6 weeks apart were usually most satisfactory. The texture of the material was also a significant factor in phytotoxicity. Coarse material that took a long time to break down was safer at higher rates. Sulfur applied in an emulsifiable concentrate was very fast acting but caused some phytotoxicity if rates were too high.

The biggest problem we had with soil acidification was the occurrence of phytotoxicity on beds that were poorly drained and had long durations (more than 1 week) of standing water after sulfur applications. Well-drained sandy sites with no standing water after applications were free of noticeable phytotoxicity on cranberries. That is, peaty soils were always problematic and sandy soil much less so. The cause of toxicity was not directly related to soil acidification but to the reduction of inorganic sulfur compounds. Under conditions of poor drainage, sulfate is reduced to sulfite and then to sulfide. Free hydrogen sulfide is toxic to roots and will accumulate under waterlogged conditions. Apparently there is sufficient sulfite or sulfate under these wet conditions to allow for toxic levels of sulfide production. Successful use of sulfur on cranberry bogs, therefore, seems limited to well-drained plantings, application timings which avoid periods of water logging, and gradual rather than rapid acidification. In conclusion, although soil acidification has its limits, well drained cranberry beds with a soil pH above 5 and where poor herbicide efficacy has resulted in an over abundance of legumes and other weeds, should be considered for sulfur applications as part of the weed management program.

Herbicide and soil pH interactions: In general, we found that, when soil acidification was combined with herbicides, much lower rates of herbicides could be used to achieve control of several weed species. There could be several reasons for this. First, some herbicides like Devrinol are reported to work better at a low pH than moderate or high pH (Johnson and Burns, 1985). Second, the suppression of weeds by Devrinol wears off early, at which time the more gradual influences of soil acidity can come into effect. Third, soil acidity may weaken the weeds making them more susceptible to herbicide damage. This latter factor may be especially important for perennial weeds.

3) Fine tuning of herbicide use.

Since we have so few herbicide options it is important that we maximize their effectiveness by the proper choice of application timing and rates, and by the best use of herbicide combinations. These recommendations are site specific so only general principles will be mentioned here. Most obvious is to use the right herbicide for the right weed. Table 1 with the matrix on which herbicide controls which weed can be used to provide that information. Herbicides last longer on peat beds but higher rates are required for weed control. On sand beds, low rates will provide control but the time frame for control is shorter. Split applications of Casoron should be considered in this case. We have had consistently better weed control with less phytotoxicity when Casoron has been applied in two or more small applications, spaced 3 to 5 weeks apart, compared to a single large dose. The second dose can be lower than the first and should be applied before bud development is very advanced. The new 1996 label revision allows for this type of modification in Casoron use. Herbicide combinations have also been successful for control of several of the more difficult weeds in Washington. This includes Casoron with Devrinol in a 1:1 or 1:1.25 ratio, or Casoron with 2,4D-G in a 5:1 ratio put out in split applications. For the worst weeds, a typical application might be a Casoron:2,4D mix at 60# total product/acre in early March followed by 40 to 50# of this mix in early April. This example is not a recommendation for Wisconsin and is used only to illustrated that numerous combinations and multiple applications can be used to improve weed control. On-farm research is suggested to optimize those rates, timings, and combinations.

Literature Cited

Johnson, B. J. and R. E. Burns. 1985. Effect of soil pH, fertility, and herbicides on weed control and quality of bermudagrass (*Cynodon dactylon*) turf. Weed Science 33:366-370.

Walker, A. and S. J. Welch. 1992. Further studies of the enhanced biodegradation of some soil-applied herbicides. Weed Research 32:19-27.

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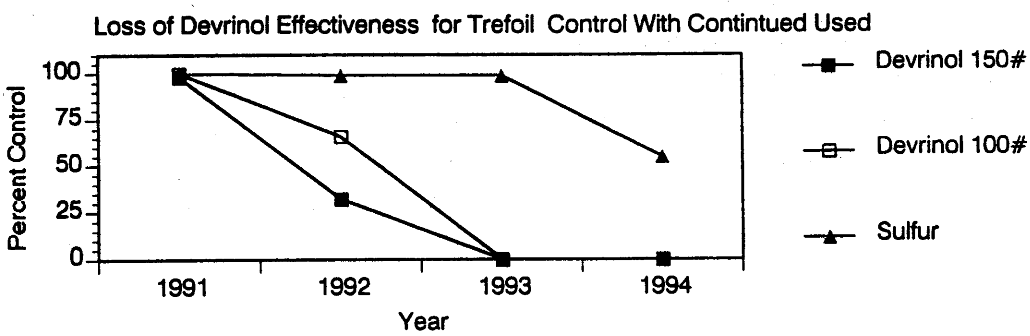

Figure 1 -- The loss of effectiveness of Devrinol and sulfur for Trefoil control with continued use. Devrinol 10 G @ 150# product/ac was applied in February and the 100#/ac rate was applied in March. Sulfur rates were 500 #/ac in 1991 three times, 500#/ac in 1992 once, 250#/ac in 1993 once and 100#/ac in 1994 once. This data demonstrates the likelihood that enhanced microbial degradation of Devrinol in cranberry beds is occurring. The data on sulfur also demonstrates how weed control can be lost when soil pH can drift upward over time as a function of not reapplying sulfur in adequate amounts to maintain a low pH.

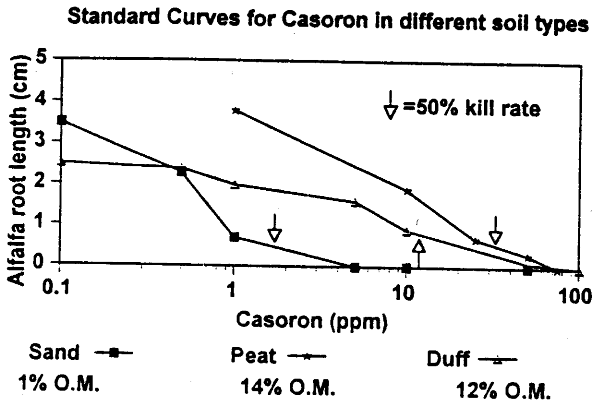

Figure 2. Standard curves (logarithmic scale) for the rate of Casoron required in different soils to prevent alfalfa root growth. Data indicate a ten-fold higher rate is required in soils with a 14% organic matter content than on sand.

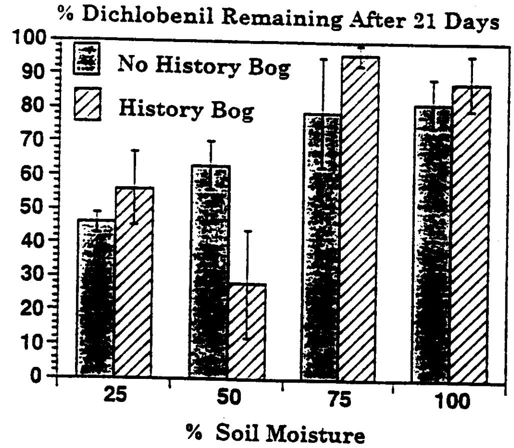

Figure 3. Effect of soil moisture on recovery of Casoron (Dichlobenil) after 21 days of incubation in two different cranberry soils--one with recent Casoron use (history bog), the other without Casoron for several years (no history bog). Twenty-five percent and 50% moisture are unsaturated soil conditions; 75% and 100% moisture are saturated soil conditions. Data provided by Dr. Allan Felsot, Food & Environmental Quality Lab, WSU- -Tri-Cities.

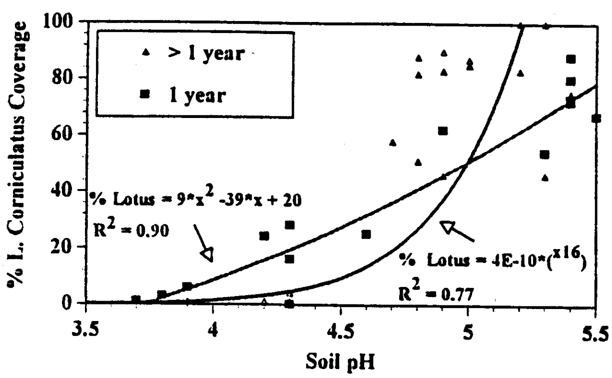

Figure 4. Percent Birdsfoot Trefoil (Lotus corniculatus) coverage in a cranberry bed as a function of the soil pH. Soil pH values after one year of sulfur treatment indicated Trefoil ceased to be a problem below pH 4.0. Soil pH values in the second year of treatment indicated that no Trefoil occurred below a soil pH of 4.5.

HERBICIDE EFFECTIVENESS ON WEEDS IN CRANBERRIES[1]

1-96

WEED FAMILY		Soil Applied Herbicides: Princep	Evital	Casoron	Devrinol	2,4-D	Postemergent Herbicides: Roundup	2,4-D	Poast	Prism[2]
Amaranth(Pigweed)	Pigweed, redroot	G	F	G	G	G	G	G	P	P
Buckwheat	Dock, broadleaf	P		G			G	G	P	P
(Knotweed)	Knotweed	G	G	G	F	F	G	P-F	P	P
	Smartweed	G	F	F-G	G	F	G	G	P	P
	Sorrel, red		G	G	G	F	G		P	P
Buttercup	Buttercup	P	P	F	G	F	G	F-G	P	P
Composite	Aster, purple			F-G	F				P	P
	Dandelion[3]	*	*	G	*P	G	G	G	P	P
	Goldenrod, western				P		G		P	P
	Groundsel, common	F	F	G	G		G	G	P	P
	Hawksbeard, bristly			G	P		G	P	P	P
	Pineappleweed	F	G	G	G	F	G		P	P
	Prickly lettuce	G		G	G		G	G	P	P
	Ragweed, common	G	F	G	F		G	G	P	P
	Salsify, western				P				P	P
	Sowthistle	G	F	G	G		G	G	P	P
	Spanish Needle (beggarstick)	G	P	G	P		G		P	P
	Tansy ragwort			G	P		G	G	P	P
	Thistle, common	*		G	P	F	G	G	P	P
	Thistle, Canada	P	G	F	P	F	F	P	P	P
Evening Primrose	Fireweed	G		G	P		G	F	P	P
	Yellow weed			F-G	P	P	G	G	P	P
Ferns	Bracken fern	P	P	F	P	P	F-G	P	P	P
	Sword fern	P	P		P	P	P-F	P	P	P
Figwort	Speedwell					P	G	F	P	P
	Toadflax			P	P		P	P	P	P
Geranium	Geranium, cutleaf			G	G		G	F	P	P
Goosefoot	Lambsquarter	G	G	G	G	G	G	G	P	P
Grass (annual)	Barnyardgrass	F	G	G	F	P	G	P	G	G
	Bluegrass, annual	G	G	G	G	P	G	P	P	G
	Bromes, annual[3]	G	G	G	G	P	G	P	F-G	G
	Velvetgrass	P	G		P	P		P	F-G	P
Grass (perennial)	Bentgrass	*	*	G	*	P	G	P	F	G
	Rice cutgrass	P	G	F	G	P	G	P	F	G
	Saltgrass	P	F-G	P-F	P	P	G	P	F-G	G
	Quackgrass	P	P-F	G	P	P	G	P	P	G

WEED FAMILY		Soil Applied Herbicides					Postemergent Herbicides			
		Princep	Evital	Casoron	Devrinol	2,4-D	Roundup	2,4-D	Poast	Prism[2]
Horsetail	Field horsetail	P	P	G	P	P	P	G	P	P
	Scouring rush	P	P-F	G	P	P	P	G	P	P
Madder	Bedstraw		G				P	P	P	P
Mint	Henbit	G	G	G	P	F	G	F	P	P
Legumes	Clovers[3]	P	P	F	F-G	P	F	P-F	P	P
	Lotus/brdsft trefoil	P	P	P-F	F-G	P	P	P-F	P	P
Mustard	Bittercress, little	F	G	G	G		G	G	P	P
	Cress, hoary	*		G					P	P
	Pepperweed	*		F-G		G	G	G	P	P
	Mustard, wild	G	G	G	G		G	G	P	P
	Shepherdspurse	G	G	G	P	G	G	G	P	P
Nightshade	Nightshade	G	G	G	P		G	G	P	P
Pink	Chickweed	G	G	G	G	F	G	G	P	P
	Corn spurry	G		G	G		G	P	P	P
Plantain	Plantain	P	G	G	P-F	G	G	G	P	P
Purslane	Minerslettuce	G	G	G	P		G	G	P	P
	Purslane, common	G	F	G	G	F	G	F	P	P
Rose	Silverleaf	P	P	P-F	*	P-F	F-G	G	P	P
Sedge	Sedge species[3]	P	F	G	P	P	F	P	P	P
	Nutsedge/yellow	F	G	P	G	P	P-F	P	P	P
Rush	Arrowgrass	P	P	P	P	F		G	P	P
	Rush species[3]	P	F-G	F-G	F	F	F-G	G	P	P
	Lousegrass/toadrush		G	G	F		G	G	P	P
St. Johnswort	St. Johnswort			G	P		G	F	P	P
Woody Plants	Alder	P	P	P	P	P	G	G	P	P
	Blackberry[3]	P	P	P	P	P	G	F	P	P
	Poison oak	P	P	P	P	P	G	F	P	P
	Salal	P	P	P	P	P	P	F	P	P
	Salmonberry	P	P	P	P	P	G	P	P	P
	Scotchbroom & gorse	P	P	P	P	P	G	G	P	P
	Willow[3]	P	P	P	P	P	F-G	F-G	P	P

[1] This chart is intended only for planning your weed control program. Weed control will depend on timing, rates, environment and stage of weed development. Use herbicides with care; always check the label before using. Apply only to plants and sites listed on the label and use only the application methods and rates listed on the label. Herbicides listed are those registered for cranberries in Washington and Oregon for 1996. It is advised to use up existing stock of Princep as use in future years may be cancelled.

[2] Herbicide registered on non-bearing bogs only.

[3] There are numerous species of this plant. Herbicide effectiveness will depend upon which species is being treated; some species may be resistant.

(*) = Seedling control only; G = good (80-94%); F = fair (60-79%); P = poor (less than 59%)