FasTrack Breeding and other Novel Approaches to Germplasm Improvement

Ralph Scorza, Ann Callahan, Chris Dardick, Chinnathambi Srinivasan
USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV

Expression of the poplar *Flowering Locus T1 (FT1)* gene

Induces:

- Early flowering
- Continual simultaneous development of flowers and fruit in the greenhouse and field

SEQUENCE OF PRODUCTION OF EARLY FLOWERING AND FRUITING PLUM

Date of plum transformation:	**July 31, 2007**	
Date of flowering in vitro:	**October 5, 2007**	**~2 months**
Date of planting rooted plants in a 6” pot In the greenhouse	**December 10, 2007**	**~2 months**
Date of flowering in the greenhouse	**January 23, 2008**	**~1 month**
Date of harvesting of fully ripe plum fruits with viable seeds	**July 21, 2008**	**~6 months**

~ 1 yr.

Date of flowering of seedlings (F1) in greenhouse	**December 10, 2008**	**~5 months**
Date of harvesting of fully ripe plum fruits with viable seeds	**July, 2009**	**~6 months**

~ 1 yr.

Fruiting cycle of clone 103

Flowering after three months in soil.

Fruit set after pollination

Ripe fruits 5 months after flowering

OPTIMIZING FRUIT PRODUCTION IN THE GREENHOUSE

How plants respond to:

- Temperature
- Daylength
- Chill regime
- Leaf stripping
- Fertilization
- Pruning

Meeting the first FasTrack breeding goal:

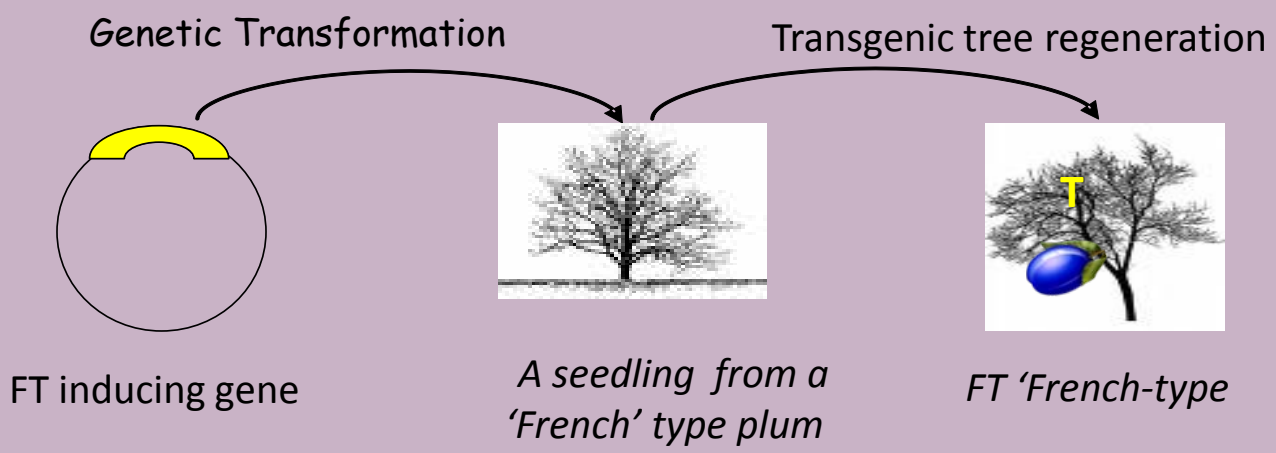
Optimizing fruit production in greenhouse.

Early and continuous flowering and fruiting allow FasTrack Breeding year-round under greenhouse conditions

“FASTRACK” PERENNIAL CROP BREEDING

STEP 1:

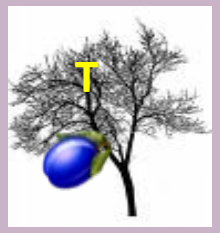
Transformation of Seedlings of ‘French’ types
with the early, continual flowering (FT) construct



STEP 2: An example

Crossing the FT line for several generations with high sugar but otherwise poor quality plums to develop sweeter plum varieties.

In each generation the highest quality FT plums with the highest sugar content are selected and used as parents

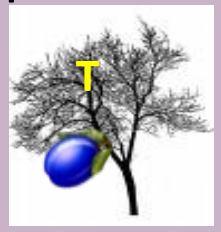


FT Plum

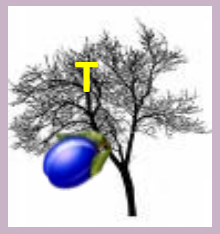
X

High sugar plum

Use markers



SELECT
Higher sugar, high quality FT trees as parents for the next generation

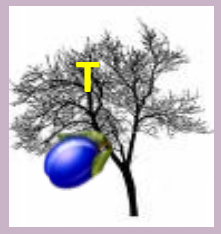


Higher sugar high quality FT trees

back **X** cross

High sugar or other high quality trait

Use markers



SELECT
Higher sugar, high quality FT trees as parents for the next generation

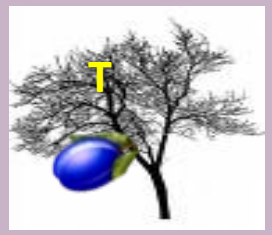
This can be carried out until the breeder has trees with fruit that have a good combination of sweetness, other flavors good size, color, etc. that also resemble ‘French’

STEP 3:

Select the best.

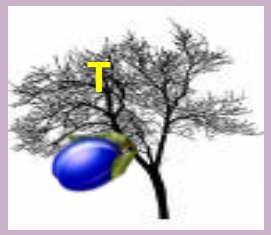
There are 4 types of trees to choose from in the last generation:

High sugar
high quality types



FT + High sugar
types

Not desirable
sugar and/or fruit type

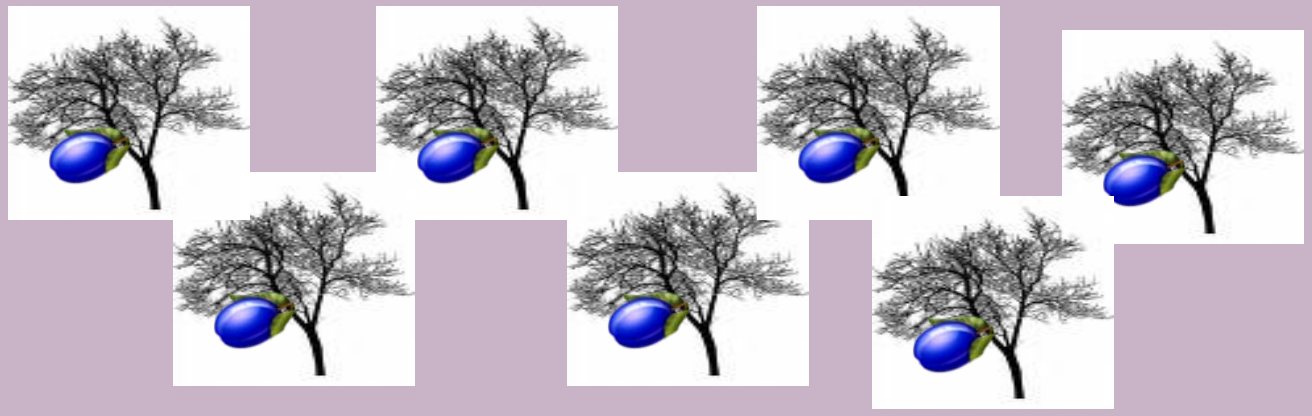


FT not desirable
sugar and/or fruit type

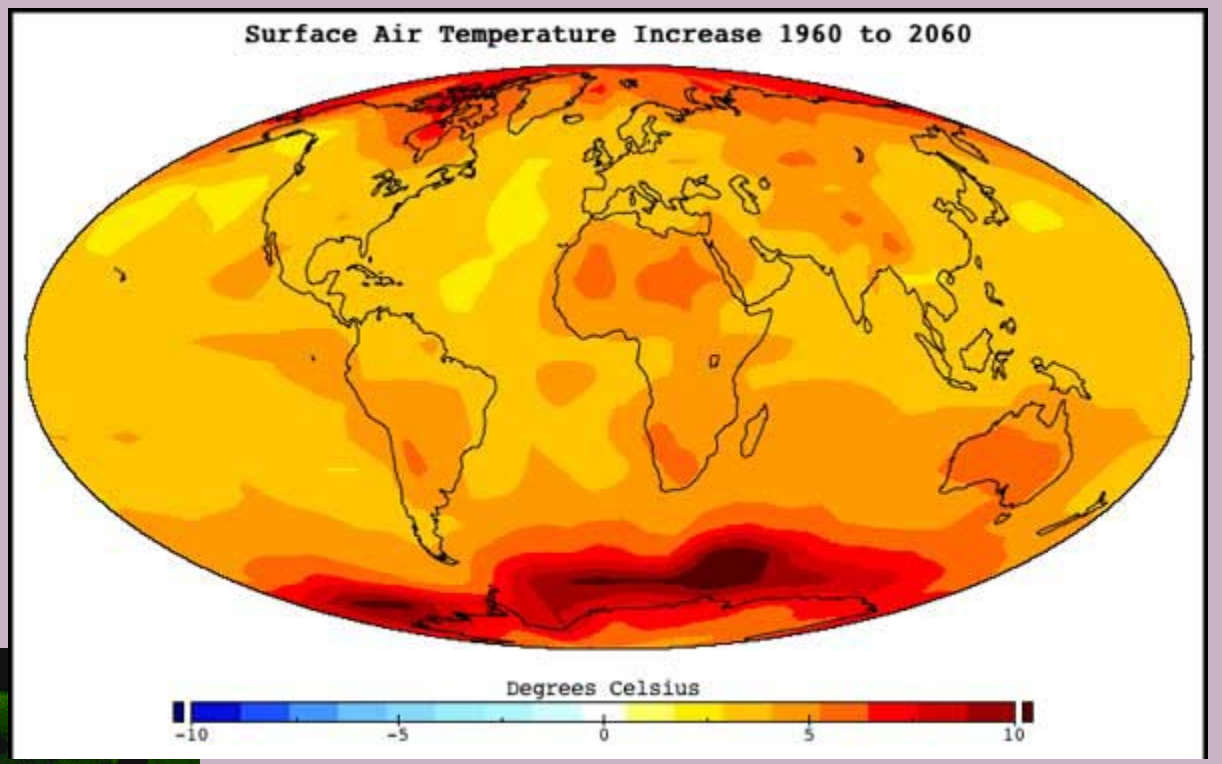
Only the high sugar, high quality, non-FT types are selected. They may become cultivars or advanced selections for further, traditional breeding

Outcome:

'FasTrack' Breeding as can, in a relatively short time, provide improved tree fruits (in this case high quality, high sugar plums). For example, considering a generation time of 4 years for plum, 3 backcross generations would normally require 16 years. FasTrack breeding would accomplish 3 backcross generations in 5 years! In the end the selected FasTrack bred trees in this example are not genetically engineered.



GENETIC ENGINEERING FOR THE RAPID DEVELOPMENT OF IMPROVED GRAPE VARIETIES TO RESPOND TO BIOTIC AND ABIOTIC STRESS RESULTING FROM CLIMATE CHANGES, EXOTIC PATHOGENS AND COMBINATIONS OF BOTH



GRAPE RESEARCH PROJECTS THAT ARE UTILIZING GENETIC ENGINEERING AND MAY BE HEADED TOWARDS THE DEVELOMENT OF GENETICALLY ENGINEERED IMPROVED GRAPE GERMPLASM OR CULTIVARS

- Localizing genes for cold hardiness in grape
- Documenting race-specific resistance to powdery mildew and applying molecular markers to pyramid resistance genes
- Powdery mildew genomics and transcriptomics
- Development of transgenic hairy root systems for grapevine functional gene assay
- Evaluating genes for potential to improve grapevine architecture
- Developing rot-knot nematode resistant rootstocks

Table 1. Selected field trials of transgenic grape from US APHIS database

Institution	Date	Goal
Cornell University	11/07	leafroll and fanleaf virus resistance
	3/00	powdery mildew and *Botrytis* resistance
University of FL	12/07	*X. fastidiosa* resistance
	12/07	Powdery mildew and *X. fastidiosa* resistance
Cornell, Geneva	6/05	powdery mildew resistance
Universita degli Studenti, Ancona, Italy	8/99	fruit growth
Bundesanstalt für Züchtungsforschung, Germany	8/00	fungal resistance
INRA, France	94, 99, 04	virus resistance

Nature Biotechnology 2008 (26:261-263).

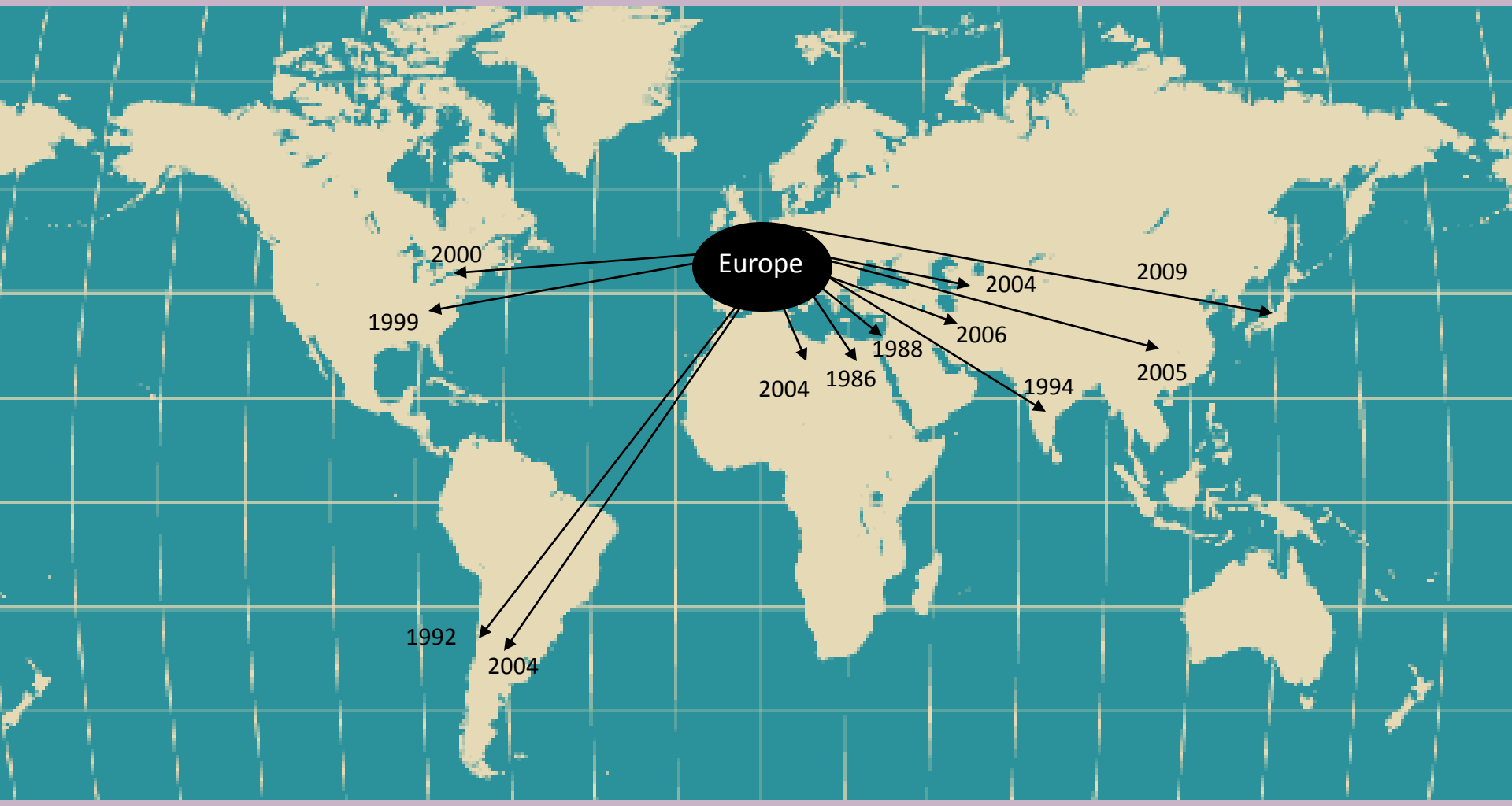
Genetic Engineering is a Powerful Tool

But what good is it if is never used to produce something useful?

Development and regulatory approval of genetically engineered PPV resistant plums

Plum pox virus symptoms

- Fruit deformation and reduced quality
- Premature fruit drop
- Leaf chlorosis
- Tree decline



Plum pox virus first identified in eastern Europe in 1918 is progressively spreading world-wide

California - *P. domestica*

99% of U.S. plum production

50-60% of world dried plum (prune) production

70,000 acres, farm gate value of $132 M

Export value $200 M

PREEMPTIVE BREEDING

Few sources of readily usable highly resistant *Prunus* germplasm are available

In anticipation of the potential spread of PPV world-wide, in 1989 USDA-ARS initiated a project to test pathogen-derived -resistance to control PPV infection

GOAL – TO PROTECT U.S. AGRICULTURE

‘HoneySweet’ R&D Timeline

Initial genetic engineering
1990

Greenhouse testing for resistance
1992-1995

U.S. field test
1995

Determination of the resistance mechanism
1995-2006

EU field plantings
1996

Field test horticultural data
risk assessments 1996-present

Regulatory submissions
2006-2007

‘HoneySweet’ U.S. Regulatory Approvals

APHIS July 2007

FDA January 2009

EPA May 2010 (conditional registration full registration expected 2011)

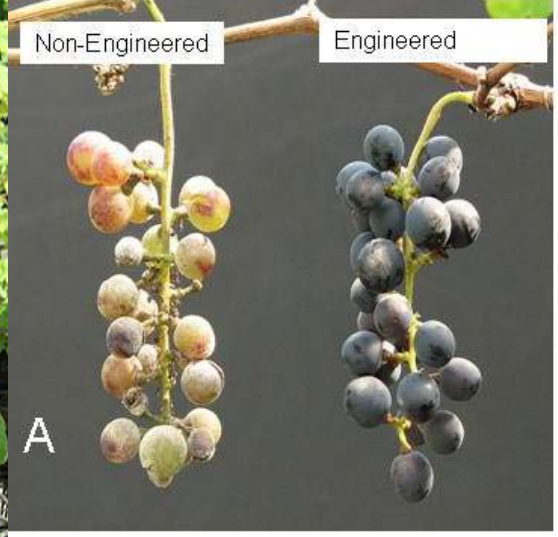
The science is there

The regulatory structure is there

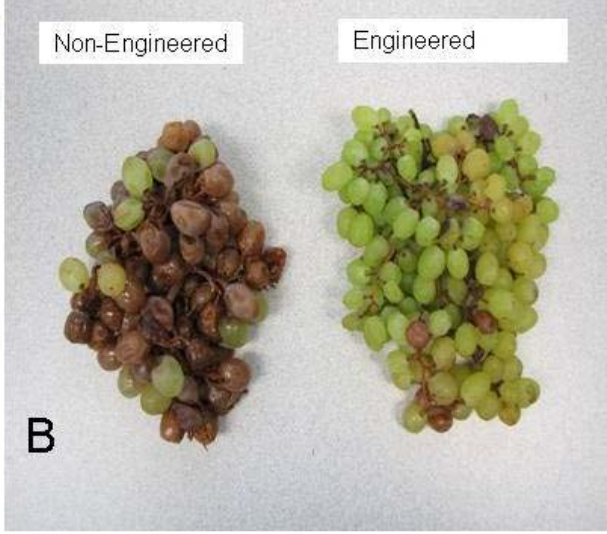
Industry support is critical

New problems require new solutions.

New solutions require new support.



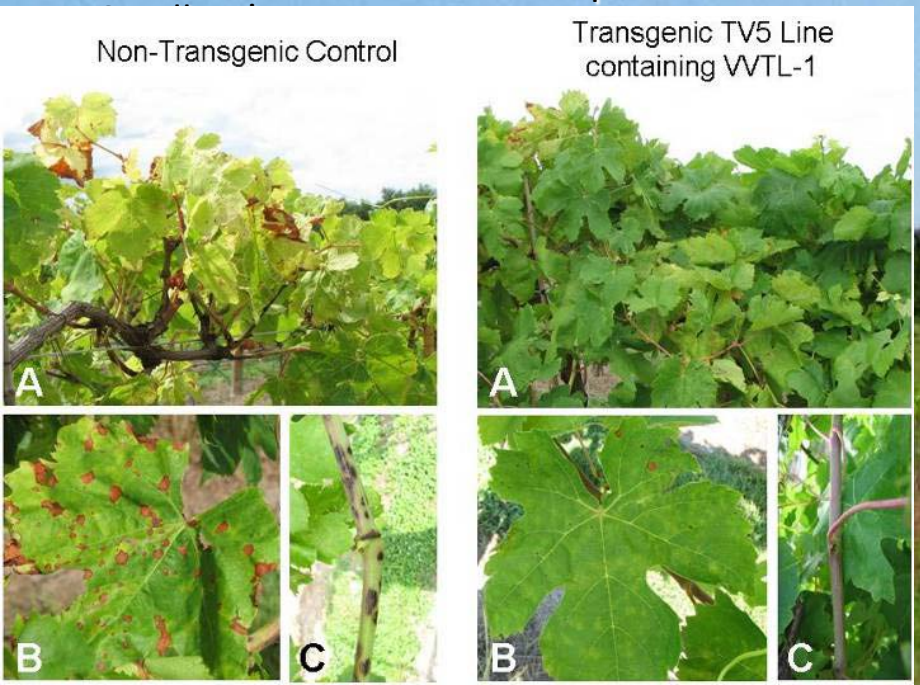
'Syrah'
Powdery Mildew Resistance



'Thompson Seedless'
Rot Resistance

Susceptible 'Thompson

Transgenic 'Thompson



OPPORTUNITIES!

courtesy of Dennis Gray

Public acceptance

The question of consumer acceptance is clouded and no one has a clear look into their crystal ball. I am optimistic.

Biotech soybean occupies more than 75% of the global soybean production , almost half of global cotton is biotech, 25% of maize and 20% of canola world-wide are biotech.

'HoneySweet' experience

2007 APHIS received 1,725 comments (1,708 negative). Most negative comments were received as cut and paste comments from a single anti-GMO website.

2010 EPA received 78 comments of which 76 were positive.

<u>An opportunity</u> - Public trust in public research institutions

I would gladly devour a plum that produced a coat protein that protects from the plum pox virus. Please keep the public informed of further positive research into genetic engineering being used to help mankind.

Please allow this genetically modified product to be marketed freely. The public will accept this disease resistant product and it will promote public discussion of genetically modified products.

Do it. Genetic engineering may sound scary, but a lot of very smart people have spent much of their time making it work safely.

Good work on coming up with new virus resistant fruit! Definitely going to make it easy for my family to keep eating healthy (and at a reasonable price!)

This shouldn't be controversial. Approve the genetically engineered plum plant. No ill effects have been seen in other genetically engineered crops.

Am in favor of this genetically-engineered strain. We need to be using these technologies in more efficient ways. All our foods are "engineered" by humans. Modern techniques are merely more efficient, and it's this efficiency which is needed more than ever, given our population pressures.

I am a private citizen who is for using science to improve crops to feed the large amount of people in the world. Please plant the resistant plum.

My primary concern with GM food lies with the industry behind their production…….

As long as the new, resistant plum trees will be available to growers without financial strings attached …..then I welcome this development.

Say no to GMOs!

The availability of GE specialty crops for the industry will depend upon:

COMMITMENT of the scientists

COMMITMENT of the institutions

COMMITMENT of the industry

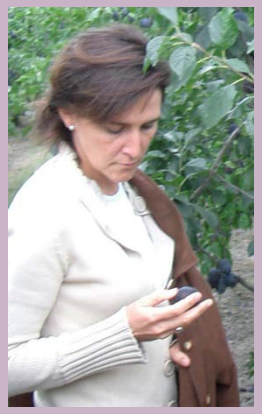
‘HoneySweet’ development has been based on these commitments

R. Scorza, ARS
A.M. Callahan, ARS
V. Damsteegt, ARS
C. Dardick, ARS
D. Gonsalves, ARS
M. Ravelonandro, France
J.M. Hily, France
M. Cambra, Spain
N. Capote, Spain
I. Zagrai, Romania
T. Malinowski, Poland
N. Miniou, Romania
J. Polak, Czech Republic
J. Kundu, Czech Republic
S. Dolgov, Russia
H. Prieto Chile
I. Kamenova, Bulgaria
S. Paunovic, Serbia

California Dried Plum Board

Black Sea Biotechnology Association

And others…….



EU-US Plum pox partnership