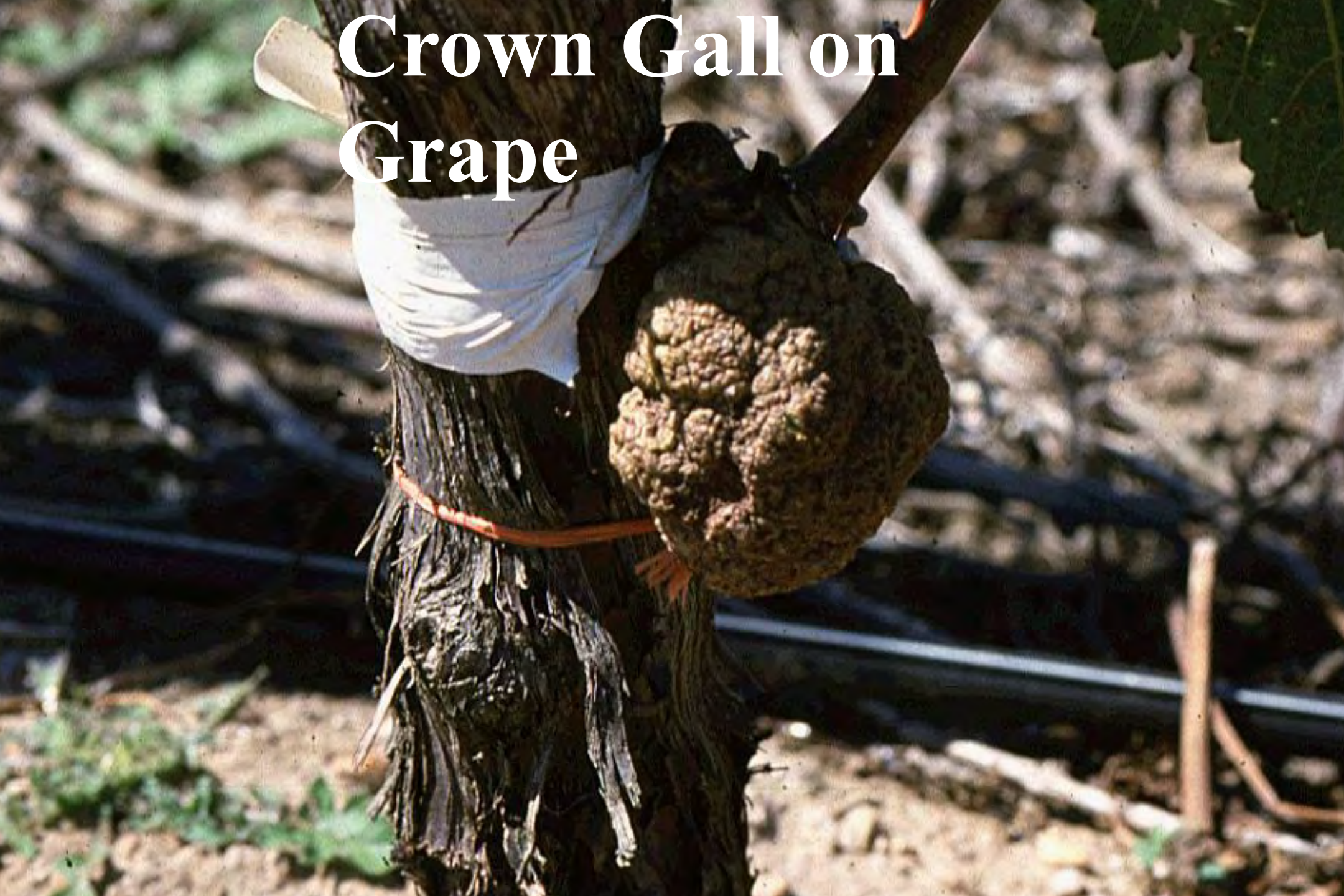
Crop Biotechnology: From Discovery to Application

R. James Cook, WSU Emeritus

Crown Gall on Grape



E.F. Smith and C.O. Townsend. 1907. A plant tumor of bacterial origin. Science 25:671-673

Agrobacterium tumefaciens
A name to become synonymous with genetic engineering

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1941-48: A "tumor-inducing principle" (TIP) produced by *A. tumefaciens*

Virulent bacteria could be killed after contact with wounded plant tissue and a gall still developed

Gall was free of bacteria

TIP initiates tumorous growth of wounded tissue at 25 C but is inactivated at 32 C.

Once the cellular transformation was complete, the gall develop at both 25 and 32 C.

1969-1986: Period of Curiosity-Driven Research

Conjugal transfer of virulence to an avirulent *A. radiobacter* making it *A tumefaciens*

Conjugal transfer of oncogenicity results from transfer of a megaplasmid

Loss of virulence during growth at 37 C results from loss of the megaplasmid

1969-1986: Period of Curiosity-Driven Research

Only a small segment of Ti plasmid DNA—the "transfer DNA or T-DNA—is required to incite tumor formation.

T-DNA is the long sought TIP and the first documentation of stable incorporation of bacterial genes into a plant

A specific component of the "Ti" plasmid is transcribed in crown gall tumors

1969-1986: Period of Curiosity-Driven Research

T-DNA is the "coreDNA"required in all Ti plasmids to maintain the transformed state, with preferred regions of the T-DNA serving as points of attachment to plant DNA

Genes for production of auxin and cytokinins and part of the T-DNA account for the galls.

Genes for production of opines (nopaline and octopine) provide C and N that only agrobacteria can use

1969-1986: Period of Curiosity-Driven Research

Short direct repeats flank T-DNA and define the borders on a nopaline Ti plasmid.

The borders are identified as 25 base-pair border repeats—all that is needed for Agrobacterium to transfer T-DNA into the plant. Similar for nopaline and octopine plasmids.

1969-1986: Period of Curiosity-Driven Research

The signal molecules from wounded plant cells that activate T-DNA transfer into plant cells are acetosyringone and a-hydroxyacetosiringone; Trigger expression of the entire vir regulon.

The virD operon encodes a site-specific endonuclease that cuts the bottom stand of the 25 base pair border repeats of both the left and right borders in the exact same place.

Development of the biological tools for transfer of foreign DNA into plants using a disarmed Ti plasmid

The Ti plasmid being so large is separated into two "mini" plasmids (a binary vector) thereby separating the vir functions from the T-DNA

Chimeric genes consisting of a gene of interest and a selectable marker are successfully expressed in plants.

Glyphosate-Resistant Crop Plants

Glyphosate targets the plant's EPSP synthase, common to green plants and microorganisms but not insects or animals

An EPSP gene from a nonpathogenic Agrobacterium introduced into the plant's genome performs all the functions of the plant's EPSP synthase but is unaffected by glyphosate

Glyphosate-Resistant Crop Plants

Necessary to test hundreds of transformants to find one that met agronomic standards.

The selected transgenic genotype is used as a parent in conventional breeding.

USDA approved for commercial production soybean, corn, canola, sugar beets, alfalfa, and cotton, starting in 1996.

“—63% of soybean growers who reduced their tillage since 1996 cited herbicide-tolerant technology as the key factor in doing so.”

Conservation Tillage Information Center
Purdue University

Insect-Resistant Crop Plants

A single gene from the insect pathogen *Bacillus thuringiensis* is used for production of an insecticidal protein.

Production of Bt toxin increased >hundred-fold by using a truncated, condon-optimized (A/T replaced by C/G) gene or a synthetic gene

Insect-Resistant Crop Plants

Corn for resistance to European corn borer

Cotton for control of boll insects

Potato for control of Colorado potato beetle
(USDA-approved; Rejected by MacDonald's)

Bringjal/eggplant in Bangladesh; 2013

Cotton Bolls

Conventional

Bt Cotton

NCFAP

In China—

Adoption of cotton varieties with *Bt*-resistance to insects has reduced pesticide use by 13 spray applications per hectare per season, saving $762 per hectare per season

(Huang et al. 2002. Science 295,674-677.)

European Corn Borer

NCFAP

Bt Corn

Insect Resistant **Insect Susceptible**

Viral Coat-Protein-Mediated Resistance to Plant Viruses

Cucumber mosaic virus in the Atlantic East

Papaya ring spot virus in Hawaii

Virtually every plant virus could be controlled by what is now recognized as post-translational gene silencing, but the technology sits on the shelf due to high costs of regulatory approval

The genetically modified papaya plants are already being credited with saving an industry that was on its way out

New York Times
July 20, 1999

OSU Virus-Resistant 'Meeker' Raspberry

Virus Susceptible

Virus Resistant

Globally, according to ISAAA

In 2014, 18 million farmers in 28 countries planted more than 181 million ha to biotech crops

On average, adoption of GM crops over the past 20 years:

- **Reduced the use of pesticide by 37%**
- **Increased crop yields by 22%**
- **Increased farmer profits by 68%**

The "Gene Revolution"

>99% involves *two* genes/gene families

—EPSP synthase gene for glyphosate tolerance

—Bt gene family for insect resistance

Rht-2 gene for dwarf growth habit in wheat—Washington

Sd-2 gene for dwarf growth habit in rice--China

The "Green Revolution"

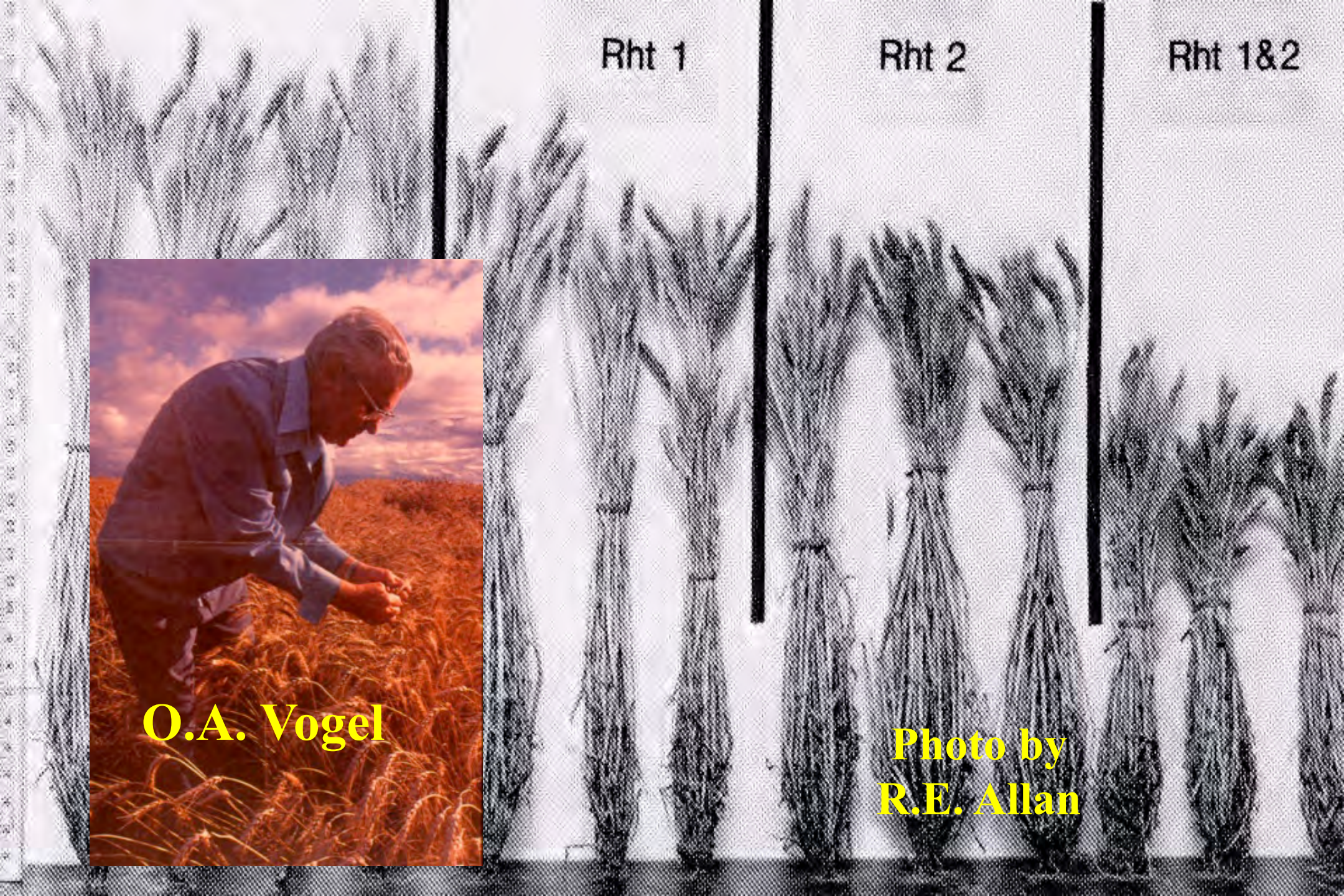
Examples of Single-gene Solutions Applied to the PNW Wheat

RHt gene family for semi-dwarf growth habit

Genes for stripe rust control

Pch1 gene from wheat relative for foot rot control

Resistance to BASF's IMI herbicide produced by chemical mutagenesis of *ALS*


Rht 1
Rht 2
Rht 1&2
O.A. Vogel
Photo by
R.E. Allan

Stripe Rust of Wheat

Puccinia striiformis f. sp. *tritici*

Susceptible

Intermediate

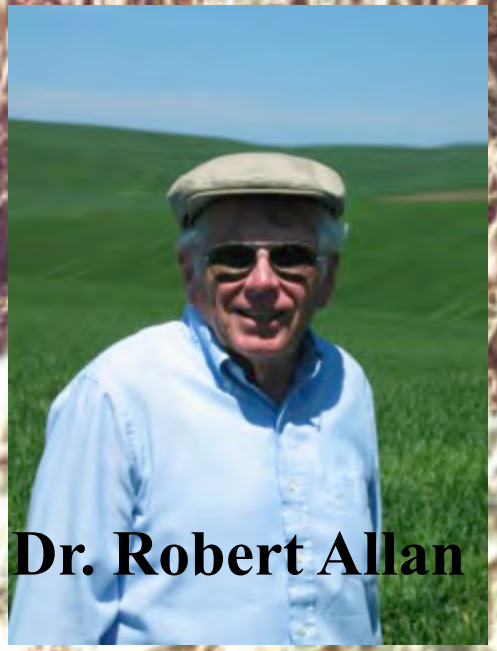
Resistant

Eyespot Foot Rot (strawbreaker)

Sprayed with a fungicide

Not sprayed

Pch1 gene for foot rot resistance



Dr. Robert Allan

'ORCF-101'

A new SWW variety and tool for grassy weed control using CLEARFIELD® technologies

Recognitions and Awards

Gene Nester and four of his PhD students and postdoctorals elected to the National Academy of Sciences

Ernest Jaworski, Robb Fraley, Stephen Rogers and Rob Horsch of the original Monsanto team recognized with th 1998 National Medal of Technology and Innovation

Mary-Del Chilton of the Nester Lab, Marc van Montegu from Ghett, and Robb Fraley from Monsanto received th 2013 World Food Prize

Thank You