AN UNUSUAL VIRUS IN TREES WITH CITRUS BLIGHT

RON BRLANSKY

UNIVERSITY OF FLORIDA, CREC

CITRUS BLIGHT

- KNOWN IN FLORIDA FOR OVER 100 YEARS; FIRST DESCRIBED IN 1874
- PROBLEM IN FLORIDA IN THE 1970'S WITH INCREASE IN ROUGH LEMON ROOTSTOCK
- THOUSANDS OF TREES BECOME UNPRODUCTIVE EVERY YEAR, RESULTING IN LOSSES IN EXCESS OF $60 MILLION ANNUALLY

WHAT HAPPENS WITH BLIGHT?

- FIRST SYMPTOMS MAY BE A ZINC DEFICIENCY IN THE LEAVES WHICH MAY VANISH AS THE TREE BEGINS TO DECLINE
- LEAVES ARE SMALL ON AFFECTED TREES WITH AN OFF-GREEN TO GRAY COLOR
- ZINC FIRST ACCUMULATES IN THE BARK AND THEN IN THE WOOD
- NEXT WATER UPTAKE IN THE XYLEM IS IMPAIRED DUE TO AMORPHOUS PLUGGING
- PRESENCE OF SPECIFIC PROTEINS IN EXTRACTS FROM AFFECTED TREES

SEARCHING FOR THE CAUSE

- POTENTIAL CAUSES SUCH AS NUTRITION, SOIL TYPE, SOIL PH, BACTERIA IN THE SOIL OR PLANT, AND VARIOUS FUNGI HAVE NOT PROVEN AS THE CAUSE

- ELECTRON MICROSCOPY FOR BACTERIA OR OTHER AGENTS IN BLIGHT-AFFECTED TREES WAS CONSISTENTLY NEGATIVE

- SYMPTOM COMPARISONS OF OTHER DECLINE DISEASES WITH BLIGHT WERE NOT THE SAME

- **ROOT GRAFTING EXPERIMENTS WITH BLIGHT TRANSMITTED THE DISEASE (PATHOGEN) TREE TO TREE AND WITH ROOT PIECE GRAFTING WAS DONE IN FLORIDA & IN BRAZIL**

- CONCLUSION: IT APPEARS TO BE A GRAFT TRANSMISSIBLE AGENT (VIRUS OR OTHER AGENT)

WHERE ARE WE NOW?

- NO ONE HAS SEEN THE CAUSAL AGENT
- PURIFICATION METHODS WERE DONE BUT DIDN'T HELP ISOLATE AN AGENT
- NO CAUSAL AGENT PROVEN OR ASSOCIATED
- **NEW GENE SEQUENCING TECHNOLOGIES NOW AVAILABLE; CAN THESE BE USED?**

SEQUENCING TECHNIQUES: WHAT DO WE MEAN?

- TERMS: METAGENOMICS, DEEP SEQUENCING OR NEXT GENERATION SEQUENCING
- THE PRIMARY GOALS OF THESE TECHNIQUES ARE TO CHARACTERIZE ORGANISMS PRESENT IN A SAMPLE AND EVENTUALLY IDENTIFY THE ORGANISM

WHAT SHOULD CITRUS BLIGHT RESEARCH FOCUS ON?

- DETERMINATION OF THE CAUSAL AGENT
- SPECIFIC DIAGNOSTICS
- CONTROL/MANAGEMENT

WHAT DID WE BASE OUR WORK ON ?

- OUR TEAM: JOHN HARTUNG, WILLIAM SCHNEIDER, AVIJIT ROY AND ME HAVE BEEN WORKING WITH CITRUS LEPROSIS VIRUS, AN EXOTIC CITRUS VIRUS IN COLOMBIA
- CITRUS LEPROSIS AFFECTED CITRUS SAMPLES FROM COLOMBIA WERE NOT DETECTABLE WITH DETECTION METHODS PREVIOUSLY DESCRIBED FOR THE VIRUS; HOWEVER VIRUS WAS SEEN IN THE ELECTRON MICROSCOPE; WAS IT A DIFFERENT VIRUS? PROBABLY
- USING NEXT GENERATION SEQUENCING WE IDENTIFIED THE VIRUS AS A NEW LEPROSIS VIRUS, SO….

SO WHAT ABOUT BLIGHT?

- WITH USDA FUNDING WE BASED OUR WORK ON LOOKING FOR VIRUS SEQUENCES FROM ROOT SAMPLES OF TREES WITH BLIGHT
- REQUESTED YOUR MONEY FROM CRDF TO IDENTIFY VIRUS SEQUENCES FROM BLIGHT TREES
- WE WERE FUNDED LAST APRIL TO COMPLETE MULTIPLE SEQUENCING AND IDENTIFY VIRUS SEQUENCES IN BLIGHT AFFECTED TREE SAMPLES

WHAT DID WE DO?

- THIS PAST YEAR WE USED SEQUENCING ANALYSIS TO LOOK FOR POTENTIAL VIRUSES ASSOCIATED WITH CITRUS BLIGHT
- WE HYPOTHESIZED THAT THE BLIGHT VIRUS WAS PROBABLY AN UNUSUAL OR NEW VIRUS
- WE LOOKED FOR FRAGMENTS OF VIRUS IN TREES WITH BLIGHT, TREES WITHOUT SYMPTOMS OF BLIGHT, AND TREES WITH SYMPTOMS OF GREENING BUT NOT OF BLIGHT

SAMPLES

- SINCE ROOT GRAFTING FROM BLIGHT AFFECTED TREES TO HEALTHY TREES RESULTED IN THE TRANSMISSION OF THE UNKNOWN BLIGHT PATHOGEN AND THE PRODUCTION OF BLIGHT SYMPTOMS WE USED ROOTS AS OUR SOURCE OF SAMPLES

PROCEDURES

- TREES DIAGNOSED AS BLIGHT OR HEALTHY USING SYRINGE INJECTION TECHNIQUE
- PENCIL SIZED ROOT SAMPLES TAKEN AND PEELED TO REMOVE THE BARK
- PHLOEM TISSUES REMOVED, TISSUE CHOPPED
- THE TISSUE WAS THEN FLASH FROZEN AND GROUND
- RNA EXTRACTED FROM THE TISSUE WAS THEN PURIFIED
- RNA-ENRICHED NUCLEIC ACID PREPARATION WAS THEN USED FOR SEQUENCING BY A COMMERCIAL COMPANY

WHAT DID WE FIND?

- 65,000 READS WERE OBTAINED AND ASSEMBLED INTO FORMS THAT WERE USED TO SEARCH THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION DATABASE.
- **EUREKA!!** WE HAD VERY SIGNIFICANT MATCHES IN THE DATABASE TO A KNOWN VIRUS, PETUNIA VEIN-CLEARING VIRUS (PVCV) **A PARARETROVIRUS**
- PVCV-LIKE REGIONS WERE ALIGNED AND PRIMERS WERE DESIGNED TO AMPLIFY THE REMAINING SEQUENCE OF THE VIRUS BY DIFFERENT MEANS.
- COMPLETE VIRAL GENOMES WERE THEN ASSEMBLED USING KNOWN METHODS AND **ENDOGENOUS PARARETROVIRUS SEQUENCES WERE FOUND IN THE CITRUS DNA**

FIRST: WHAT IS A PARARETROVIRUS ?

- THESE VIRUSES ARE CALLED PARARETROVIRUSES BECAUSE OF THE WAY THEY REPLICATE (BY REVERSE TRANSCRIPTION OF RNA TO DNA).

- GENES OF SOME MEMBERS OF THIS VIRUS FAMILY ARE FOUND IN THE GENOMES OF FLOWERING PLANTS. CALLED ENDOGENOUS PARARETROVIRUSES.

- ENDOGENOUS PARARETROVIRUSES CAN EXPRESS VIRAL PARTICLES, BE INFECTIOUS AND CAUSE SEVERE DISEASES IN PLANTS

- THESE VIRUS GENES ARE GENERALLY NOT PRESENT AS FUNCTIONAL VIRUS IN THE PLANT GENOME BUT MAY BE SCRAMBLED, DELETED OR EVEN SHORTENED.

- HOWEVER THEY ARE OFTEN PRESENT IN MULTIPLE COPIES

- THEY ARE TRANSMITTED IN VARIOUS WAYS SUCH AS INSECTS, SEED, POLLEN, GRAFTING, AND CROSSES BETWEEN CERTAIN GENETIC LINES

A PARARETROVIRUS EXAMPLE

- AN IMPORTANT BANANA VIRUS, *BANANA STREAK VIRUS* (BSV) WAS ALREADY KNOWN AND CHARACTERIZED
- VIRUS SYMPTOMS DISCOVERED IN THE PROGENY OF CROSSES BETWEEN CERTAIN GENETIC LINES; STRANGE SINCE THE PLANTS USED FOR THE CROSSES WERE CONFIRMED TO BE VIRUS FREE
- PASSAGE THROUGH TISSUE CULTURE CAUSED THE VIRUS SEQUENCE IN THE BANANA GENOME TO BE ACTIVATED & PRODUCE REPLICATING VIRUS THAT THEN CAUSED DISEASE
- SPECULATED THAT IN THE PERENNIAL AND CLONALLY PROPAGATED NATURE OF THE CROP ALLOWED FOR THE PROLONGED ASSOCIATION OF THE VIRUS AND THE HOST AND ALLOWED FOR THE INTEGRATION OF THE VIRAL GENOME INTO THE HOST GENOME.

FACTORS FOR INDUCTION OF EPRVS TO FORM EPISOMAL VIRUS

- GENOME HYBRIDIZATION, TISSUE CULTURE, AND ABIOTIC STRESSES SUCH AS TEMPERATURE AND LIGHT REGIMES, DROUGHT STRESS, GRAFTING, AND PROLONGED CULTIVATION WITH REGULAR TRIMMING OR WOUNDING

OTHER STUDIED PARARETROVIRUSES

- *KALANCHOE TOP SPOTTING VIRUS* TRANSMITTED BY BOTH POLLEN AND SEED
- *COMMELINA VEIN MOTTLE VIRUS* IS TRANSMITTED TO PROGENY THROUGH SEED
- *PETUNIA VEIN CLEARING VIRUS* TRANSMITTED THOUGH THE SEED OF PETUNIA
- *TOBACCO VEIN CLEARING VIRUS* TRANSMITTED BY SELF-FERTILIZATION OF *N. EDWARDSONII*, BUT NOT TRANSMITTED BY GRAFTING, APHIDS, OR MECHANICAL INOCULATION
- *DAHLIA COMMON MOSAIC VIRUS* NOT TRANSMITTED BY APHIDS; SEQUENCES WERE FOUND INTEGRATED IN THE DAHLIA GENES
- *BANANA STREAK VIRUS*

PROPOSED CONTINUED RESEARCH (ON CRDF FUNDED LIST)

- ***OBJECTIVE 1. GENERATE A COMPLETE SEQUENCE FOR BLIGHT ASSOCIATED VIRUS***

- ***OBJECTIVE 2. DEVELOP A HIGHLY SPECIFIC ASSAY TO DETERMINE WHEN THE VIRUS IS ACTIVE***

- ***OBJECTIVE 3. USE THE DEVELOPED ASSAY TO SCREEN A LARGE NUMBER OF TREES FROM BLIGHT AFFECTED AREAS IN FLORIDA***
  - USE SYMPTOMATIC TREES TO DETERMINE WHAT TISSUES CAN BE USED FOR ASSAY; DETERMINE IF THERE IS A SEASONAL COMPONENT TO VIRUS ACTIVATION.
  - DETERMINE VIRUS PRESENCE AND BLIGHT SYMPTOMS ON 100+ TREES FROM MULTIPLE LOCATIONS
  - DEVELOP A DIAGNOSTIC ASSAY TO IDENTIFY INFECTED TREES BEFORE FULL SYMPTOMS OCCUR

- ***OBJECTIVE 4. TRANSMISSION TESTS TO DETERMINE IF THE VIRUS IS THE CAUSAL AGENT OF CITRUS BLIGHT***

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