# Special Research Report #302: Plant Breeding & Genetic Engineering Attribution of Tospoviruses in Floral Crops

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### BACKGROUND

Tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (INSV) are species in the *Tospovirus* genus, and it is the only genus in the family Bunyaviridae. Hundreds of plant species, including weeds, and crops such as tomatoes, peppers, potatoes, flue-cured tobacco, peanuts. Many floral crops are among the hosts affected by TSWV in both monocots and dicots. The insect (10 thrips spp.) that transmits these viruses is also infected. Despite conventional control measures, the global movement of ornamentals is contributing to the dispersal of tospoviruses. Thus they continue to be a significant threat to the floral crop industry. INSV remains the most frequently detected virus in floral crops (personal communication, Agdia). Consequently, it is critical to devise additional methods to find and eliminate the sources of the virus by developing a

system for virus Attribution, i.e., the act of assigning the origin of a cause.

## Viral genome and its significance

The genome of TSWV has three RNA segments: the large (L) segment, the medium (M) segment, and the small (S) segment.

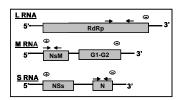


Fig. 1 Genome Organization

The genome segments code for all the important functions that permit the virus to infect the host. Since the hosts are typical for a specific geographic area, we hypothesized that the viral sequence may also be similar and, thus, indicative of viral origin. This concept has been applied to animal viruses, e.g. influenza.

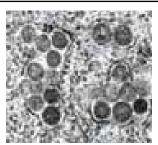


Fig. 2 Virus Particles

## MATERIALS AND METHODS

More than 70 samples infected by TSWV and 37 samples infected by INSV were obtained from our network of collaborators from five continents. The plant tissue or extracts were adsorbed in Immunosticks (Agdia) and/or FTA membranes (Whatman). RNA was extracted and RT-PCR segments were generated for sequence analyses. We developed an Optimal Sequence Alignment (OSA) system, based on statistically selected segments of the genome (arrows in Fig.1) to generate a phylogeny representative of the entire genome.

#### **RESULTS**

The evolutionary analyses (Fig. 3) with the five coding regions of the viral genome, revealed that TSWV populations have a geographic origin. This was attributed to founder effects, that includes five regions in the world: Eastern and Southern

Europe, Japan/Korea, Hawaii, California and the Southeastern USA. Thus far, for INSV isolates, the analyses of isolates from five continents (Asia, North America, South America, Europe, and Oceania) showed that the virus (Fig. 2) clustered in to two well defined groups. Group I contained the majority of isolates with a large geographic distribution. Group II contained isolates from of isolates, with a large geographic distribution. New Zealand, Japan, and one isolate from Germany. Our results also confirm the monophyly of INSV isolates and suggest that they may be undergoing a similar evolutionary pathway as TSWV.

### IMPACT TO THE INDUSTRY

- The industry has the ability to detect the various populations of INSV and TSWV.
- 2. They also have a system to determine the origin of isolates responsible for outbreaks of the virus.
- 3. A source of high quality INSV antiserum was developed and adapted for long distance surveys of tospoviruses.

### REFERENCES

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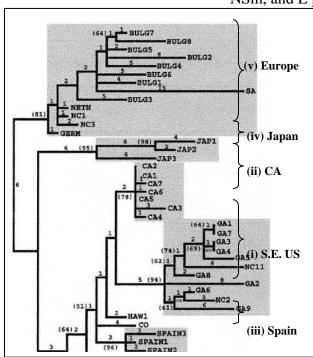


Fig. 3
Evolutionary Analysis of TWSV Populations

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