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PA Soybean Board Final term Report

**Distribution of soybean vein necrosis virus in Pennsylvania and its impact on plant growth**

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Soybean vein necrosis is an important disease transmitted by tiny insects called thrips (Thysanoptera: Thripidae). The causal agent of this disease is the soybean vein necrosis virus (SVNV) belonging to family Tospoviridae and genus Orthotospovirus. Orthotospovirus are negative sense single stranded RNA viruses. It is generally accepted that orthotospoviruses are not transmitted by seed, but SVNV is the only known tospovirus that can be transmitted through the seed, making SVNV a potential threat to soybean breeding and seed production (Groves et al., 2016). Infection of the seed results in reduction in germination percentage, seed hundred weight, and oil content (Groves et al., 2016). This virus is transmitted by different thrips species viz., Soybean thrips (*Neohydatothrips variabilis*), *Frankliniella fusca*, Western flower thrips (*Frankliniella occidentalis*), and *Frankliniella tritici* (1). SVNV is reported in Canada, USA and Middle East (Egypt). We submitted a project in 2017 to determine soybean vein necrosis disease incidence in Pennsylvania farmers' fields. In this project we focused on:

- 1) Determining the disease incidence in different soybean-producing counties in Pennsylvania
- 2) Determining the disease severity in different counties of Pennsylvania
- 3) Thrips abundance on soybean crops at different stages of soybean plant development in different counties of Pennsylvania
- 4) Determination of impact of soybean vein necrosis virus on yield and yield quality parameters

**Objective 1: Determining the disease intensity and severity**

We selected six counties in Pennsylvania viz., Centre County, Lancaster County, Lebanon County Crawford County, Armstrong County and Columbia County. These counties were selected because their wide geographical distribution will allow us to understand the virus incidence in different

soybean growing areas. We surveyed these counties and randomly picked three farmer fields and collected 60 leaves, 20 per farmer field and recorded disease severity on scale of 0-4. Collection of leaves was done in the month of July and was repeated in October 2017. The collected leaves were placed in zip lock bags and the leaves were brought to the laboratory to determine the disease incidence percentage and number of thrips per leaf under microscope. *Neohydatothrips variabilis* density per leaf was determined in different counties of Pennsylvania through examining the trifoliolate leaf. For this purpose thrips number was counted on 20 leaves per replicate. Trifoliolate leaves were selected at random, placed in the ziplock bags and thrips density was counted in the compound microscope under the lab conditions.

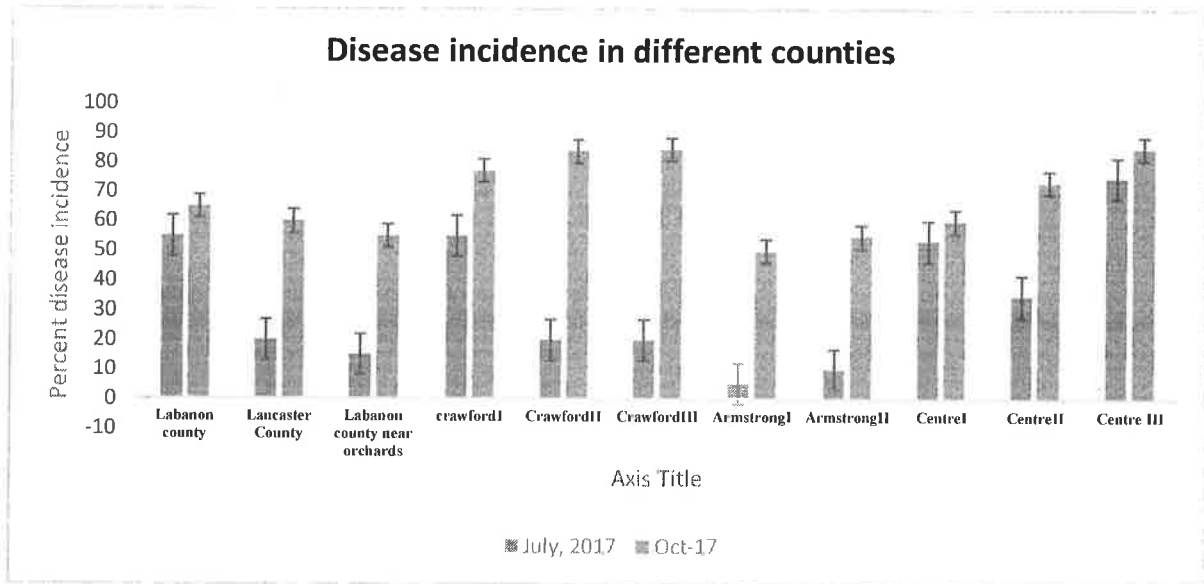
Visual scoring criteria for disease was as follows.

- 0 no disease incidence
- 1 minor vein necrosis
- 2 minor vein necrosis plus blotching
- 3. Minor vein necrosis plus blotching plus major vein necrosis
- 4. Disease spread to majority of leaf surface

## **Results:**

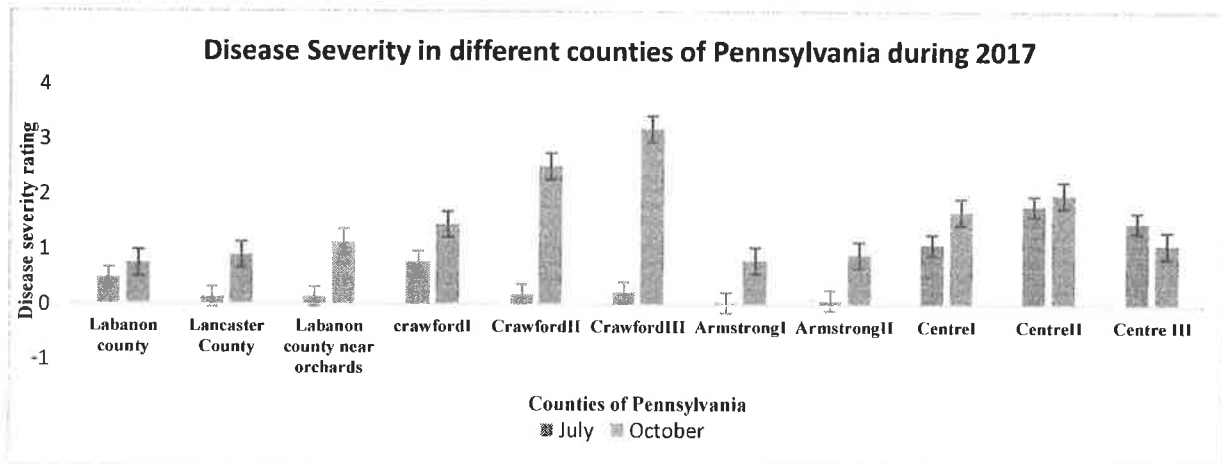
### **Disease incidence in different counties of Pennsylvania**

SVNV was generally distributed throughout the state. The disease incidence is defined as percentage of infected plants in a particular location. Incidence ranged from 5 to 70% in July and 45-80% in October showing that the disease incidence increased with time. This may be related to the thrips seasonal dynamics and the progression of disease with time. The results are presented in Fig 1.



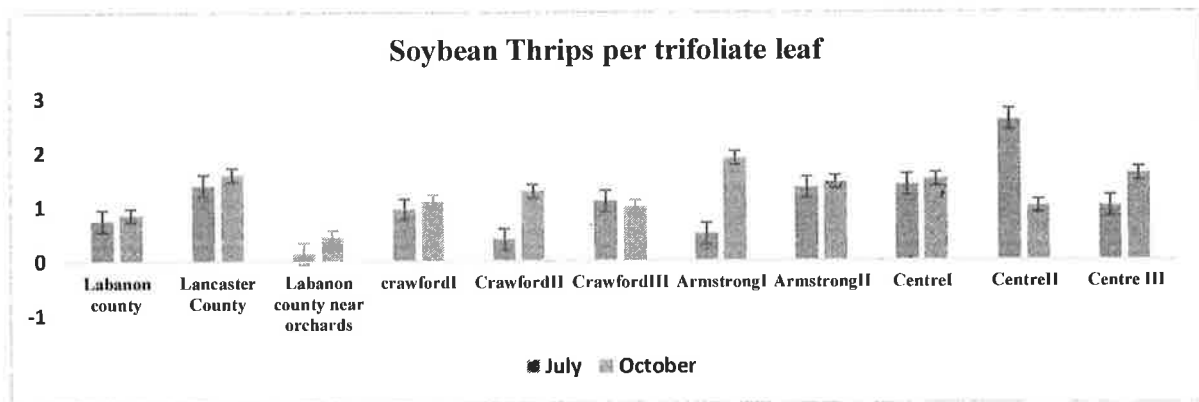
**Disease severity in different counties of Pennsylvania:**

Disease severity is defined as the 'area of a sampling unit (leaf) affected by disease, expressed as a percentage or proportion of the total area' (leaf), while disease incidence is defined as the 'number of plant units sampled that are diseased expressed as a percentage or proportion of the total number of units assessed. Disease severity was determined on the scale of 0 to 4. The results are shown in Fig 2. The disease severity increased with time. However, the increase was predominantly in the Crawford county and Centre county.



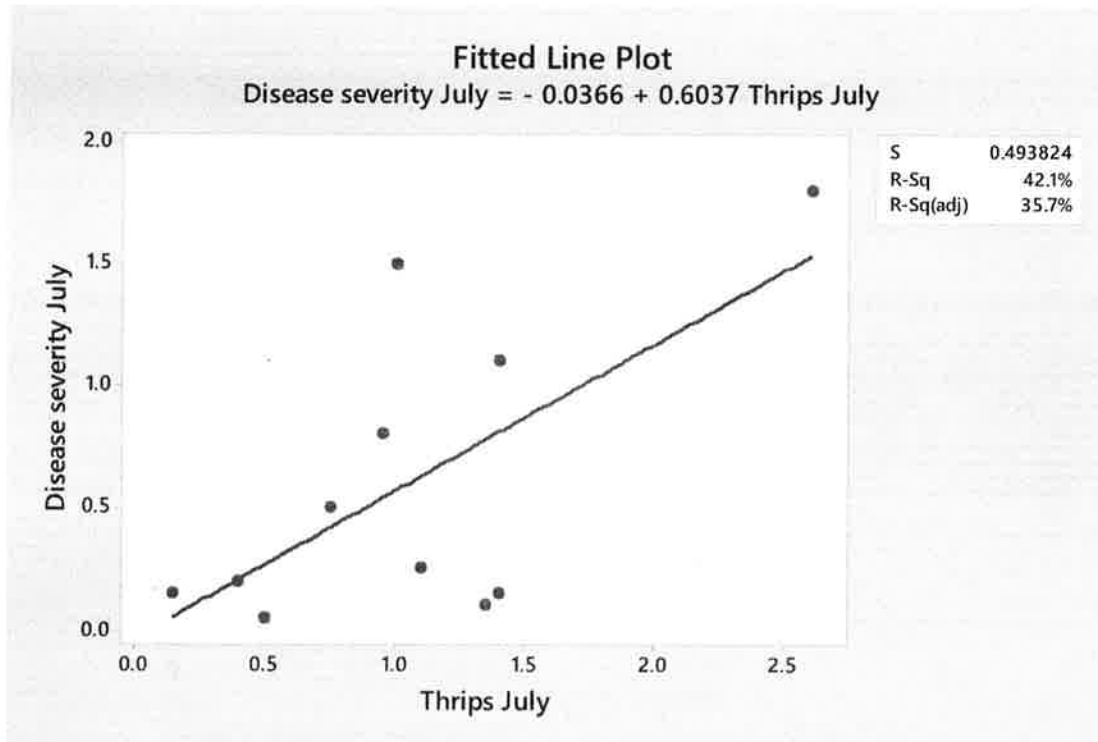
**Thrips density per trifoliolate leaf in different counties of Pennsylvania:**

Thrips density increased with time in different counties of Pennsylvania. However, the Thrips density was higher in the Centre county and Armstrong county and Lancaster county but thrips density was lower in the Lebanon County, and Crawford county. This may be related to the geographical location of these counties. Because Centre county and Armstrong county have low temperatures as compared to the Lebanon county and the Lancaster county could mean that the thrips are more common in the lower temperature zones compared to the higher temperature zones. Results are shown in Fig 3

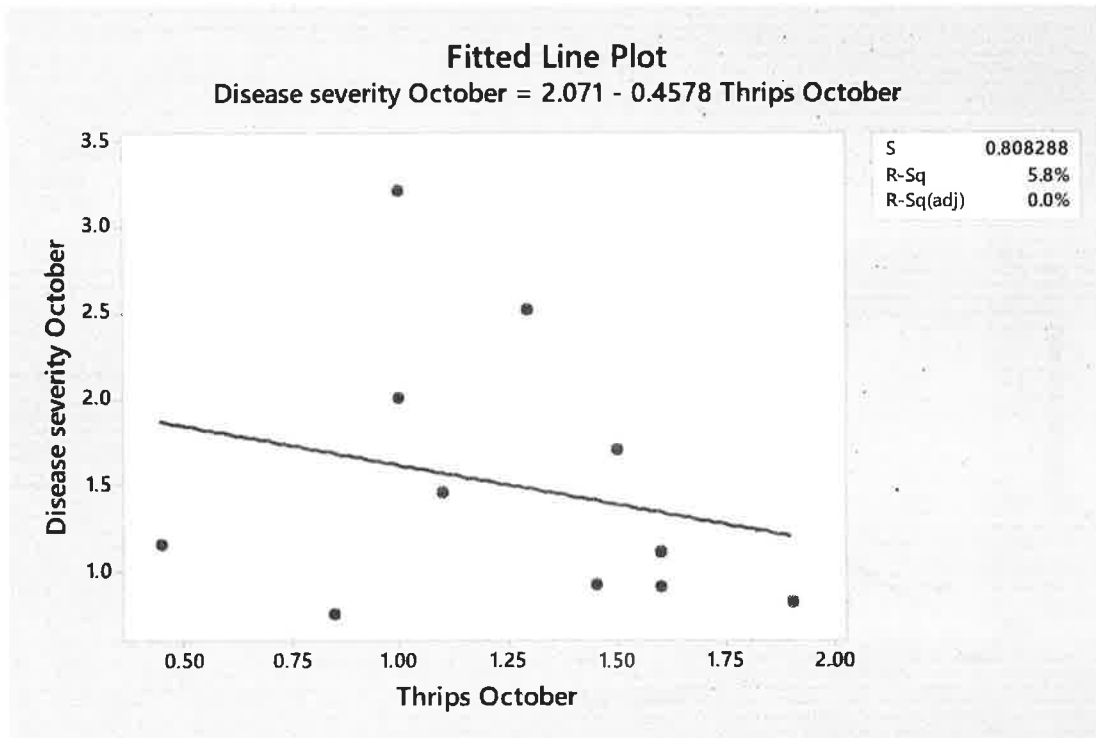


### Relationship between thrips density and disease severity

Consider that there is a good correlation between thrips in July and disease severity. However there is no correlation between thrips in October and disease severity. Keep in mind that disease severity is higher in October. Is there a saturation effect? During the month of October the crop is reaching maturity. All the nutrients are transferred to beans and the beans are attaining full size and leaves are falling off. Due to this the thrips migrate but the disease intensity is higher because virus titers saturate in the leaves.



**Fig 4 : Regression of Disease severity in July and Thrips abundance per plot in different counties**



**Figure 5: Fit of regression line in Thrips abundance in October and Disease severity**

**Objective 2: Perform growth chamber and greenhouse studies to determine yield and quality impacts**

**Preliminary Experiment:** we conducted a preliminary experiment in the growth chamber at  $25 \pm 2^\circ\text{C}$  to understand how the soybean vein necrosis virus affects the growth and yield parameters of plants. The experiment consisted of two treatments

T1 Plants having no infection as no insect was released on those plants and no mechanical inoculation was done.

T2: Mechanical inoculation was done using a buffer virus suspension. Infected leaves were ground in buffer medium and the solution was rubbed on the leaf surface to transfer virus by creating mechanical injury

The experiment was conducted on 25 plants per treatment. Leaves from infected plants were ground in a buffer with a pestle and mortar. The solution was rubbed on healthy leaves at v1 stage. Both treatments were present in same chamber, but no insects were present, so plant to plant transmission was not possible. Infected plants showed the SVN symptoms after 30 days of virus

exposure. Yield parameters were evaluated when plants reached maturity, approximately two months after the plants started formation of pods in the growth chamber. The number of nodes, pods, dry pod weight and yield per plant were observed. The preliminary results revealed that the infected plants had lower number of pods, nodes, plant height, dry pod weight and yield per plant.

**Detailed experiment:** Based on the preliminary experiment, a more detailed experiment was carried out to evaluate the effects of thrips and SVN infection on plant health and yield. We focused on determining plant growth and yield effects due to thrips and virus individually and together. For this purpose we used three groups of plants viz., infected (carrying virus and thrips), healthy (carrying thrips but no infection), control having no thrips or virus. The plants were placed in rearing cages which were thrips proof to avoid the movement of thrips from one cage to another. Also, the experiment was done in two different growth chambers. Healthy thrips chamber did not have the infected thrips cage. Results are expressed in Fig 6.

Results revealed that plant height, number of leaves (nodes), beans per plant, bean weight and number of seeds per bean pod were significantly higher in control plants compared to the healthy plants having only the healthy thrips and infected plants (having infected thrips and virus). Plant height was significantly higher in the control plants compared to the healthy and infected treatment. Number of nodes was statistically similar in the control plants and healthy treatment while it was significantly lower in the infected treatment. Number of beans per plant was statistically similar in control and healthy plants and was significantly lower in the infected plants. Bean weight was significantly higher in the control plants and was significantly lower in the healthy and the infected plants. Number of seeds per bean was significantly higher in the control plants compared to healthy plants and was significantly lowest in the infected plants (Fig 7)

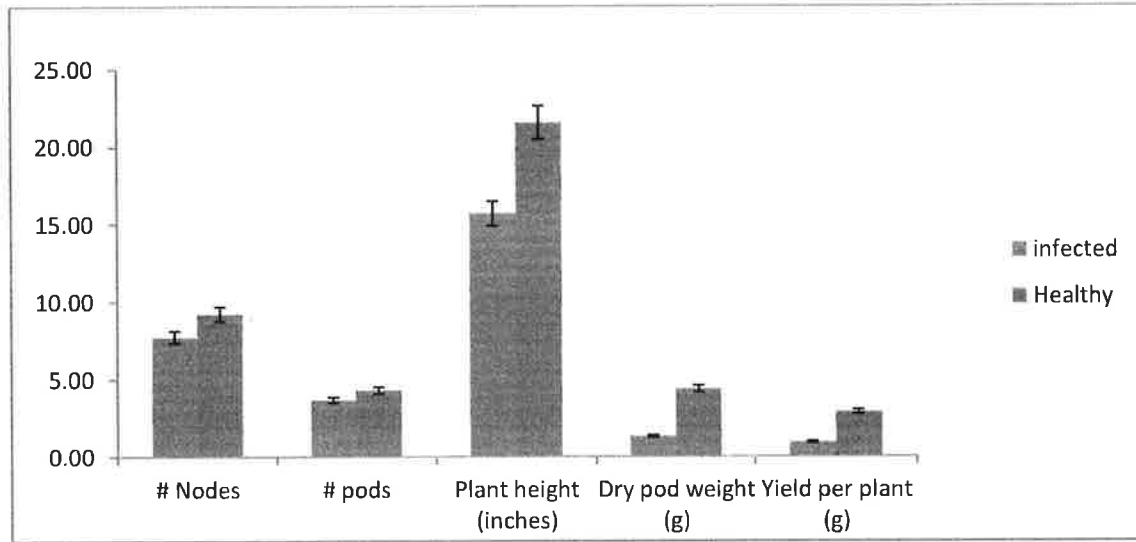
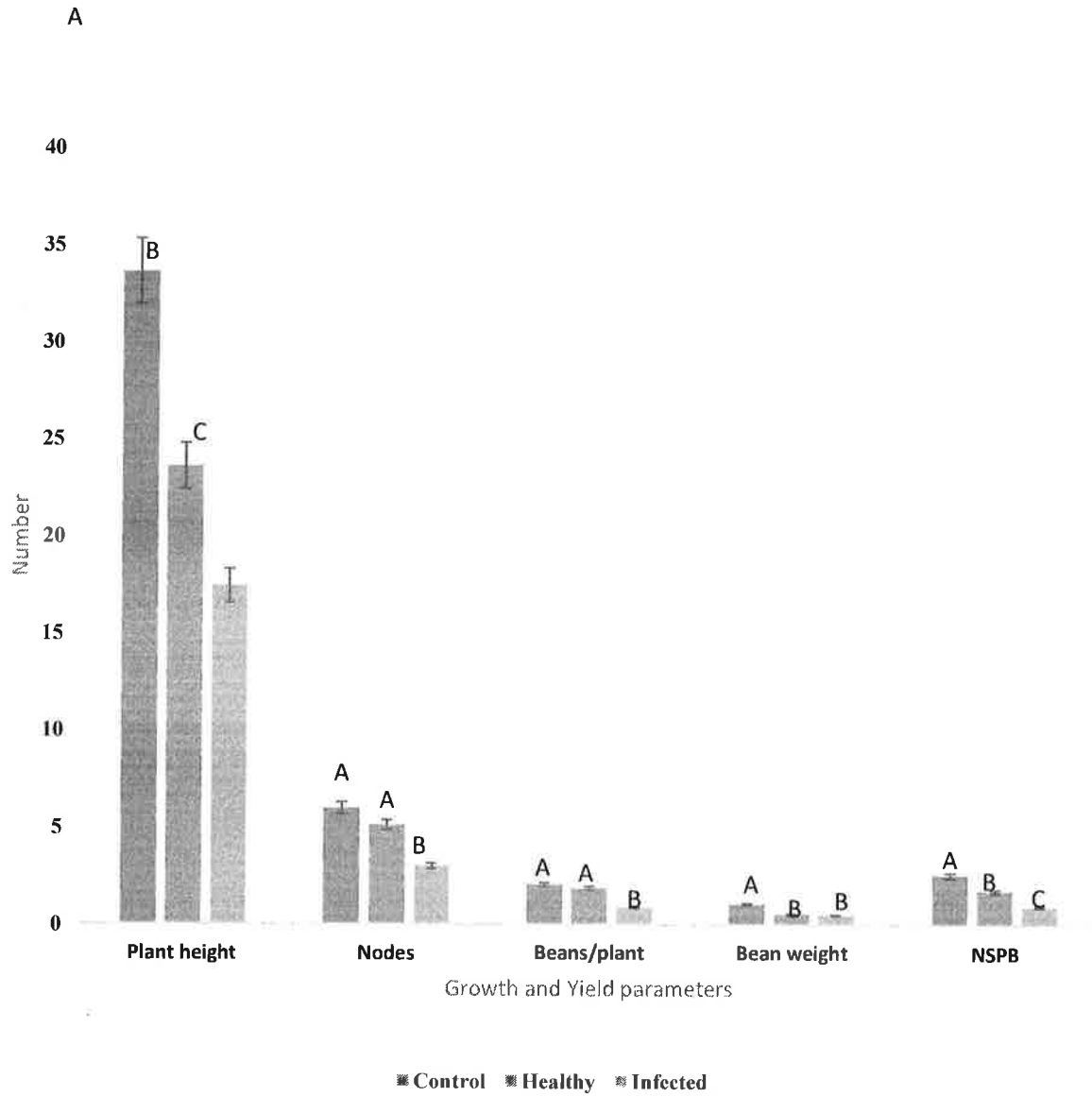


Fig 6: Effect of mechanical inoculation of virus on the nodes, number of pods, dry weight, yield per plant in infected and healthy plant.



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### Effect of virus on the plant yield and functions



## **Fig 7: Effect of virus on number of nodes in control, healthy and infected plants.**

### **Discussion**

The purpose of these experiments was to determine disease intensity, severity and thrips infestations in different counties of Pennsylvania as well as the impact of SVN on plant growth and productivity. The primary questions the study addressed was “is variation in disease incidence linked to geological location parameters?”. Is disease incidence linked with the vector density? Does virus cause damage to the plants, and if it does, then which plant growth parameters are affected?. Viruses adopt different mechanisms to spread in plants and insect populations. The type of virus and its relation with host confer the ability of virus to spread in wide range of climates (2). During the survey we found that the virus and vector was present in different geological areas, but even the diseased plants were tolerant and showing yield, which means that diseased plants are able to survive and can themselves be season-long virus reservoirs able to spread disease. Groves et al (2016)(3) found that the virus can be transmitted through the seed at 6% rate, which means that not only management of vector but also enhancing the strategic policies for seed evaluation for virus presence in predominant varieties before the distribution of next generation seed is needed. In the present studies we found that, in the growth chamber conditions, virus can cause reduction in yield of the infected plants, specifically reducing plant height, number of nodes, number of pods and number of seeds per bean. We infer that the disease may cause yield losses and broader studies are needed to characterize this. One strategy can be the use of plant selection forces to combat the disease spread. This strategy was first observed in plants that evolve different chemical and morphological characters to avoid the attack of thrips which results in the reduced disease intensity levels. The future studies on the use of plant biochemical signals which deter thrips abundance are needed.

On the basis of the present results, we propose that the virus may affect plant physiology e.g plant photosynthesis, stomatal conductance, carbohydrate contents and starch contents. We also propose that since plants combat the attack of diseases, insects and pests through the evolution of plant morphological and physiological features which reduce the replication of virus and propose hindrance to insects feeding and oviposition, so identification of these factors through varietal selection may help to reduce disease and pest attack. We also propose that plant metabolites may repel insects to some extent. Optimizing various approaches including antixenosis, antibiosis,

preference and tolerance we can reduce the extent of damage caused by vector and vector borne viral disease.

### **References**

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3. Groves C, German T, Dasgupta R, Mueller D, Smith DL. Seed Transmission of Soybean vein necrosis virus: The First Tospovirus Implicated in Seed Transmission. *PLoS ONE*. 2016;11(1):e0147342.

