

Brassica Stunting Disorder (BSD): Has the culprit been identified?

An update on the latest research results.

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Over the last four years, Brassica farmers across diverse regions of South Africa (SA) have observed a new disease called 'Brassica stunting disorder'. The disease affects various *Brassica* species, but cabbages (*Brassica oleracea* var *capitata*) appear the most affected. The disease is characterized by stunted plants, flattening and sometimes purpling of the leaves, side shoot development, vascular discoloration in the stem and/or midrib of leaves, poor root development, low yield and low quality of the final product, thereby reducing the market value. In order to develop effective disease management practices and ensure continued sustainable production of *Brassica* crops in SA, the disease causal agent(s) needed to be identified. Since 2013, researchers at the University of Johannesburg has investigated the disease in a research project that is funded jointly by the National Research Foundation and several industry members, including Bayer, Klein Karoo Seed Marketing, Sakata, Starke Ayres, Syngenta and the Seedling Growers Association of South Africa. We report here on the progress up to date.

During 2014, an infection trial was carried out within open fields and insect-free tunnels in the Brits area. It was determined that BSD is not seed, soil or water-borne and is caused by a biological agent (i.e. pathogen) that is transmitted by a flying insect. The information generated in the field trial also narrowed down the possible list of insect vector species to various species of aphids, leafhoppers and/or whiteflies.

The next step was to identify the BSD causing pathogen. Genetic material isolated from infected and non-infected control plants were compared by next-generation sequencing (NGS) using an Illumina HiSeq sequencer, in an attempt to pinpoint the genetic material of the pathogen present only in symptomatic cabbages. Although a large number of microorganisms were identified, genetic material from only one virus species were common to all infected samples analysed and absent from the control plants. Analysis of the genetic data identified the virus as an isolate of *Turnip yellows virus* (TuYV) (family *Luteoviridae*) (Figure 1). These results were confirmed with the use of another molecular detection technique (reverse transcription-polymerase chain reaction (RT-PCR)) that is specific for the detection of Luteoviruses. Following the identification of TuYV in diseased *Brassica* plants, the above mentioned RT-PCR was used to detect the TuYV in diseased *Brassica* plants that have been collected from various regions across SA between 2013 and 2015. TuYV was detected in all symptomatic cabbage plants collected from the Limpopo, Gauteng, North West, Kwazulu Natal, Eastern Cape and Mpumalanga Provinces. This is the first report of TuYV in South Africa on a crop, and indicates that there is a clear association between TuYV and *Brassica* stunting disorder.

During 2015, a follow up trial was performed in the same area in Brits in order to evaluate the influence of shade netting tunnels on infection incidence, to determine the host range of BSD and lastly, to identify the specific insect vector responsible for BSD transmission. When comparing the cabbages grown in the open field with those grown inside the insect-proof tunnels (covered by thrip netting), little or no infection was seen inside the tunnels (0.3%) and about 52% of the plants growing in the open field were infected (Figure 2). The plants grown in the shade net covered tunnels also had about 48% infection. The results again confirmed that the BSD is caused by a flying insect-transmitted pathogen and that shade net covering was not effective at reducing infection. In an attempt to determine the host range of BSD, eight *Brassica* species were also included in the infection trial. Five species, including broccoli (*Brassica oleracea* L. var. *botrytis* L.), cauliflower (*Brassica oleracea* L. var. *botrytis*), kale (*Brassica napus*) and canola (*Brassica napus*) showed typical BSD symptoms, but chinese cabbage (*Brassica rapa*), mustard (*Brassica rapa* L.) or oilseed radish (*Raphanus sativus*) did not. Using the RT-PCR technique, TuYV was also detected in all the symptomatic cabbages and other hosts screened in this trial, but absent from all the uninfected plants.

With the help of the TuYV detection technique, an attempt was made to identify the BSD insect vector. During the 2015 trial, predominant insect species occurring in the field were collected on three separate occasions. With the help of Mr. Michael Stiller from the ARC Biosystematics division, these insects were separated into three different groups, including aphids, plant and leaf hoppers and whiteflies, and placed inside cages with cabbage seedlings (Figure 3). These plants were monitored for symptom development. About 45 days later, typical BSD symptoms were observed on the cabbage plants what were caged with the aphid insects, whereas no symptoms were seen on the cabbage plants caged with the plant and leaf hoppers or the whiteflies (Figure 4). The presence of TuYV in the diseased cabbage as well as inside the aphids, was also confirmed using the molecular detection technique. In order to identify the specific aphid species responsible for TuYV transmission in this experiment, aphid samples were send for identification at the ARC Biosystematics division. Mr. Ian Miller identified the aphids as *Myzus persicae*, more commonly known as the peach-potato or green-peach aphid (Figure 5, 6).

The identification of TuYV in association with BSD in South Africa has been surprising as this is by no means an unknown virus in terms of Brassica crops. *Turnip yellows virus*, formerly known as Beet western yellows virus, is a single stranded RNA virus of the genus *Polerovirus* (family *Luteoviridae*). It is spread by a wide range of aphid species, but *Myzus persicae*, the peach-potato or green-peach aphid, is regarded as the main vector. The virus is very common in oilseed rape crops, but also readily infects brassicas, including broccoli, cabbage, cauliflower, Brussels sprouts, turnips, swede, kale. It also infects lettuce, beans and many weed species. The virus has been found widespread in Europe, the UK and recently in Australia, in areas of intensive brassica production, where in some years, all plants in commercial crops are infected. However, unlike with BSD in South Africa, in the UK and Europe TuYV infection often goes unnoticed as the virus rarely causes clear symptoms. The virus does however dramatically reduce yields, especially when

plants are infected early in the growing season. Attempts at controlling infection in Europe and Australia, especially on *Brassica napus* (canola), include control of broadleaf weeds that act as virus reservoirs, manipulation of sowing dates to avoid exposure at the seedling stage to peak aphid flights, the use of more resistant varieties and the use of a imidacloprid seed dressing to provide early control of the aphid vector during the vulnerable seedling growth stage.

In conclusion, while it has clearly been shown that TuYV are associated with *Brassica* stunting disorder and are transmitted by the green-peach aphid, further studies are required to prove that this virus is the etiological agent of disease and responsible for the symptoms seen in association with BSD. During 2016 we intend to produce an infectious clone of the South African TuYV isolate in order to further investigate its association with BSD.

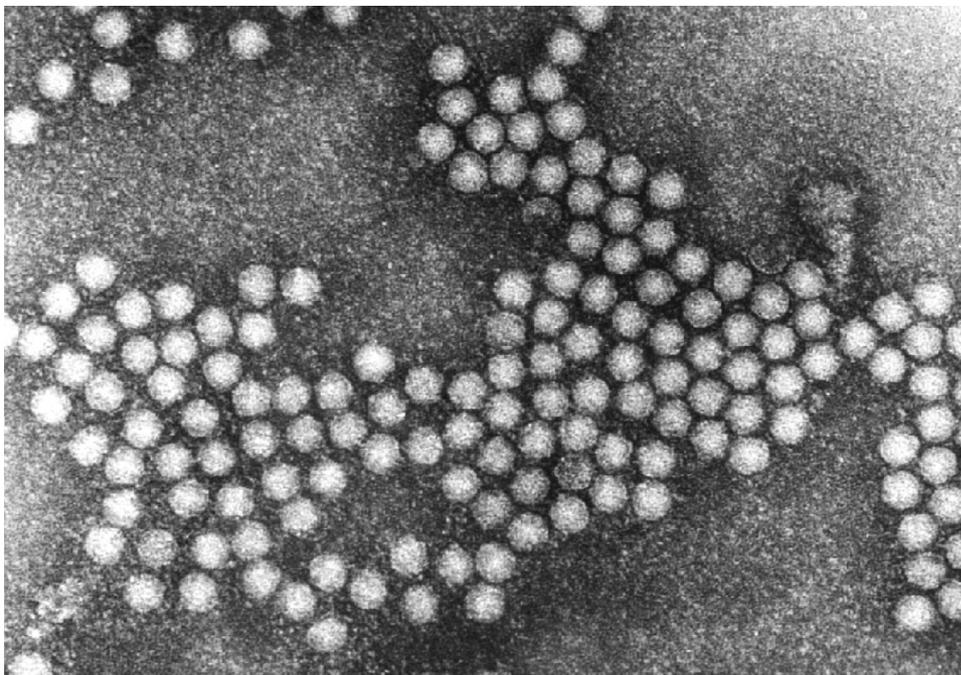


Figure 1. Electron micrograph of virus particles of *Turnip yellows virus* (<http://pvo.bio-mirror.cn/>)



Figure 2. The shade net and insect-proof tunnels used in during the 2015 trial in Brits.

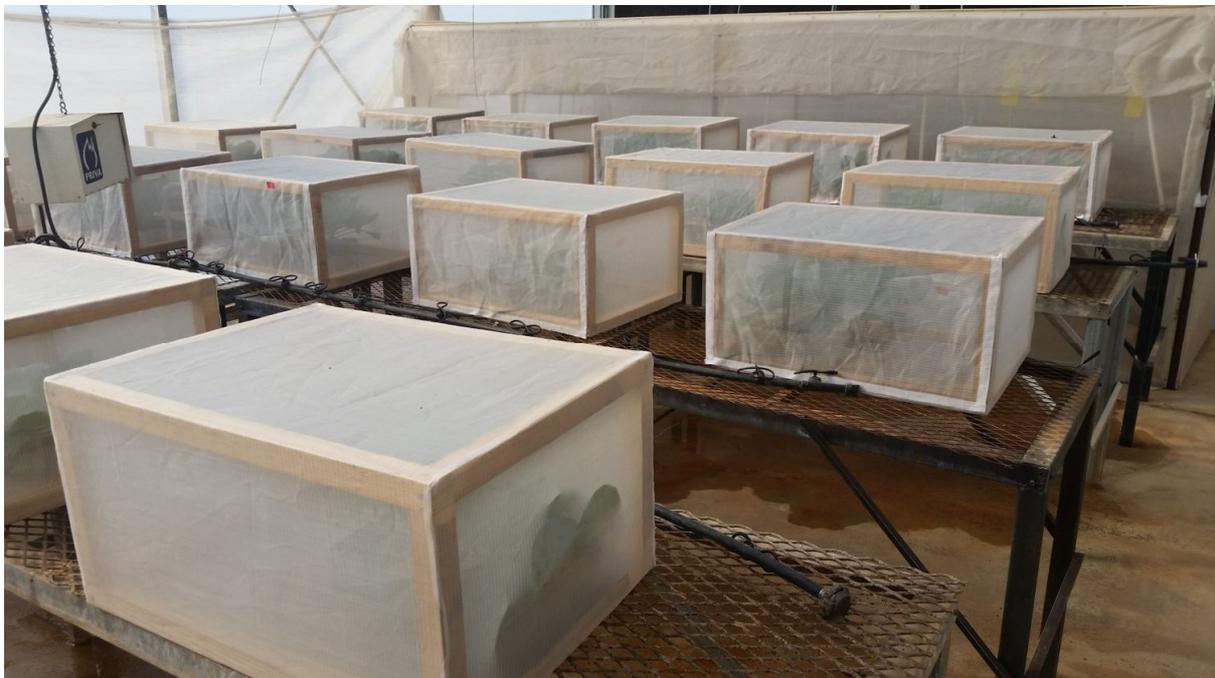


Figure 3. Cages with aphids, plant and leaf hoppers and whiteflies used in the insect transmission trial.



Figure 4. Infected cabbage (left) and healthy cabbage (right) showing the effective transmission of TuYV by aphids in the insect transmission trial 45 days after collection of the insects in the field trial in Brits.



Figure 5. Winged adult and nymphs of *Myzus persicae* visible on a cabbage leaf used in the insect transmission trial (http://aphid.aphidnet.org/Myzus_persicae.php).



Figure 6. *Myzus persicae*, the peach-potato aphid, the most important vector of TuYV (http://aphid.aphidnet.org/Myzus_persicae.php).