Hosta Virus X – Testing to Prevent its Spread

Hosta Virus X (known as HVX) has unfortunately been a problem in the hosta community for quite some time. It was first identified by Dr Lockhart in 1996 and has subsequently been found in hostas throughout the world. It is clearly a danger for commercial hosta cultivators, collectors and gardeners alike. Whilst probably all hosta enthusiasts and specialist suppliers within the UK are aware of HVX, many generalist gardeners and nurseries are not. Even within the hosta community, suppliers and enthusiasts can and should be doing more to prevent the spread of the virus.
Unfortunately we don't have a grasp of exactly how big an issue HVX is currently within the UK. An important first step would be to quantify the problem, by estimating its prevalence and spread. I believe that detection, and therefore testing of plants, must be placed at the heart of this effort.

Understanding HVX

The AHS, with partial funding from other organisations including the BHHS, has conducted some valuable scientific work in furthering the understanding of the virus; in particular how it spreads and how to protect plants against it.
The AHS has produced a leaflet which details their findings so I won't reproduce all of them in this article. It can be found on the internet at the following address:
http://www.americanhostasociety.org/Education/HVX_Brochure_Revised_Dec_2013.pdf
However, I will take a moment to highlight a couple of them. Whilst the virus is transmitted by 'sap to sap' contact with an infected hosta, this contact can also be indirect, for example via garden tools. HVX has been shown to survive for at least 3 weeks, and probably a lot longer, on such tools. To prevent infection, tools must be washed thoroughly - not just soaked - in a disinfectant such as a 10-20% solution of household bleach.
HVX has also been shown to survive for a period of 2 years in soil which previously contained an infected plant. For how much of this time the virus was active in living material, such as broken roots, and how long it survived outside of a living host isn't known, but it seems unlikely that the roots survived for the full 2 years. This implies that HVX, like several other known viruses, can survive in a dormant state for relatively long periods of time outside of a living host. So, as much soil as possible should be replaced before a new hosta is replanted in the same site as a previously infected one.
Good advice frequently given is to avoid buying infected hostas in the first place. Whilst precautions can be taken - for example visual inspections of newly acquired stock, buying from specialist nurseries, avoiding bare-root field grown divided plants where cross-contamination is a significant risk factor - I'm sceptical that any supplier can guarantee every hosta they sell is virus free. Indeed I, myself, fell foul of a specialist UK nursery a couple of years ago. I found a total of at least 7 infected plants in 2 batches of hostas I bought from them.
Further good advice is to destroy any plants which appear to be infected. Don't take the risk of spreading the virus throughout your stock of plants. Whilst I wouldn't disagree with this in principle, I also think that eliminating doubt and determining whether or not a plant is infected should be the preferred option where possible. Furthermore, the simple fact that plants can be infected for up to 2-3 years without showing any symptoms, but remain contagious, may well negate the efforts of even the most vigilant 'observer and destroyer'.

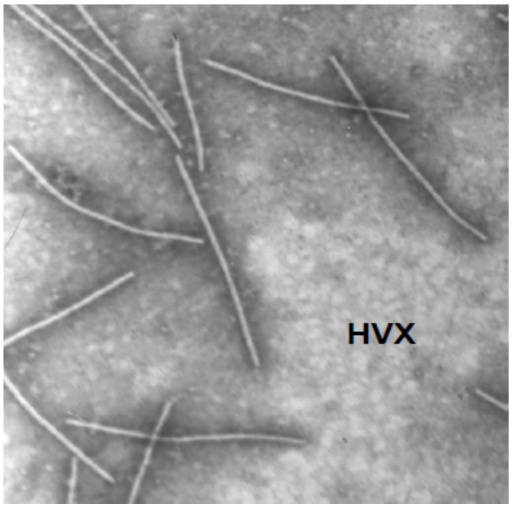
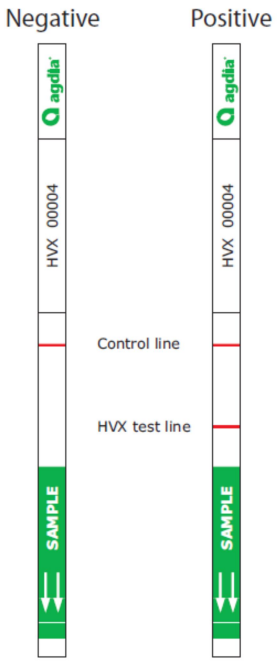


Fig 1. The HVX virus under microscope
Photo: Dr. L.F.Salazar

This brings me on to the main topic of my article: testing for HVX. There are many practical, economic and emotional reasons why testing for HVX is important for the commercial and hobby hosta community alike. If your favourite, most expensive, more sought-after hosta looks like it might be infected, wouldn't you want to know for sure before consigning it to the bonfire? As a retail nursery, wouldn't you also want to know whether your supplier has sent you a batch of infected stock; in fact, wouldn't you want to randomly batch test all of your new supply for HVX?
In the cold light of day, this makes perfect sense, but I fear that some suppliers are unwilling to acknowledge or accept the issues.
For example, large-scale European production has facilitated the supply of hostas to the general gardening public at ever more affordable prices. However, it has been widely reported that some members of the Dutch wholesale trade regarded a 10% infection rate amongst their plants as acceptable. Thankfully, this situation seems to have improved significantly now, with better plant handling procedures and some testing being carried out, particularly prior to Tissue Culture production. However, we can't afford to be complacent; more can and should be done.
Finally, as I mentioned above, with the possibility of an infected plant remaining visually free of symptoms for several years, testing is often the only way of identifying HVX at a relatively early stage.

<u>Methods of Testing for HVX</u>

Fig 2. *The ImmunoStrips® showing positive and negative test results*



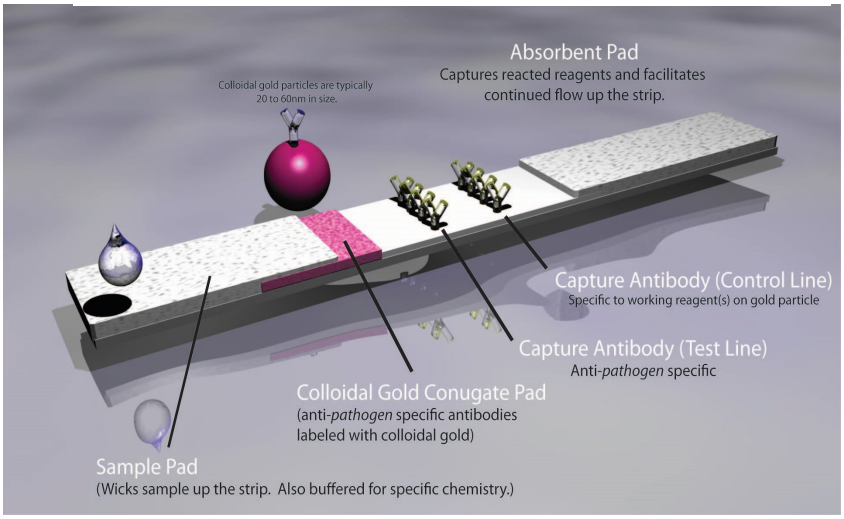
So, is there an easy, inexpensive way for us all to determine whether or not our hostas are infected? The answer is yes – well, pretty much yes.
Agdia, a leading American provider of plant disease diagnostics, released a 'rapid test strip' for the detection of HVX in 2008. The test is based on similar diagnostic principles to ELISA (Enzyme-Linked Immunosorbent Assay), a well-known and extensively used method for detecting all sorts of viruses in humans and animals as well as plants. It is designed to be used in the field so is faster and more user friendly than ELISA. The product is part of Agdia's Immunostrip® range of kits which cover tests for a total of 35 viruses in wide a range of plants such as Tobacco mosaic virus.
The test works as follows:
A small amount of plant material, such as a section of leaf or root, is added to a buffer solution supplied in a plastic bag. The material is crushed into the solution and a test stick, the size of a small pencil is slid into the bag. The solution travels up the stick by capillary action (wicking) and the presence of the virus is indicated by a line on the stick changing colour. There is also a control line to indicate that the solution is working effectively and that the test has

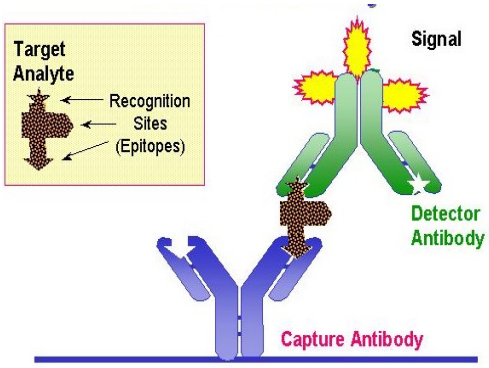
been conducted properly.
Here's a link to a video of a test demonstration https://www.youtube.com/watch?v=xgr-7-tFYFA

The Science behind the Test

For those of you interested in the science behind the test, I will explain.
The test is based on the reaction between antigens (a virus) and antibodies (a protein produced to fight the antigen). When the antibody comes into contact with its corresponding antigen, it attaches itself to the antigen. The Immunostrip® contains two small lines of antibodies, the test line and the control line, termed the 'capture antibodies'. As the name implies, the role of these antibodies is to attach themselves to the HVX antigen if it is present in the plant material which is now in the solution. Unfortunately, there is no visible change to the strip of antibodies when the antigens become attached to them, so no indication would be given on the test strip that the plant material contains HVX. To achieve such a change, the strip has had another antibody (known as the detector antibody) already added to it within the 'Congugate pad'. And here's the clever bit: the detector antibody in turn has a coloured particle attached to it (shown as a large pink sphere in fig 4 and a gold 'flame' in fig 5). If HVX is present in the plant material, the HVX antigen will attach itself to the detector as it moves up the test strip onto the conjugate pad.

Fig 4 : Schematic of the test strip





Now we have a detector antibody with an HVX antigen attached at one end and a coloured particle at the other. Finally, because it attaches itself to a different part of the antigen than the detector antibody, the capture antibody is still able to attach itself to the HVX antigen as it passes the test line. So, at the test line, we have a 4-way sandwich shown in fig 5. The antigen is held between the capture antibody and the detector antibody and the coloured particle (or signal) is attached to the outside of the detector antibody. As more detector antibodies become attached to the capture antibodies at the test line, the coloured particles become visible. They show themselves as a red line. Finally, the control line, which is above the test line, contains another antibody which is able to capture the detector antibody and its signal even without the presence of the antigen. So, as long as the solution is viable and the test is conducted properly, the control line will always change colour.

Fig 6: *An infected Piedmont Gold*

The test is claimed to be 99% effective and I've found it to be very easy to use. The kits are portable and will keep for about a year after purchase, provided they are stored in the right conditions (essentially in a fridge). They can be purchased directly from the French subsidiary of Agdia, Agdia-Biofords. The one potential drawback, which leads to my slight qualification of their endorsement, is that at around £4.40 per strip/test, the kits may be a little expensive for some gardeners, particularly generalists. If there is enough interest from our members though, the society may consider processing a large order to save on postage and may even be able to arrange for a bulk discount. Please let me know by e-mail (andrew@frestonhouse.co.uk) if you are interested in purchasing any of the test strips.

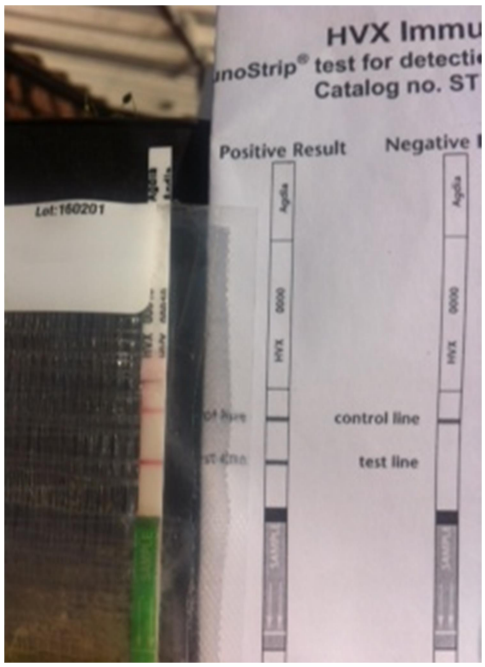


Fig 7 : *Test strip for the Piedmont Gold*

Another way in which our Society may be able to help both its members and the general gardening public in reducing the chances of them purchasing infected hostas, is to organise and run a supplier accreditation scheme. This could be based on random testing of plants sourced from suppliers who wish to be accredited. Suppliers testing negative would be accredited. Whilst accreditation wouldn't completely guarantee that any plants purchased from these suppliers would be virus free, it would non-the-less give some certainty and reassurance in this regard.

<u>Reducing the costs of testing</u>

There are ways of reducing the unit cost per plant tested, based on the fact that the strips are very sensitive to the HVX virus. The recommended amount of plant material to use for the test is 0.15g. This approximates to an area of leaf about the same size as a 10p coin or a single thin root a little shorter than the diameter of a 10p coin. Within reason, using material from more than one plant at once will not materially affect the reliability of the strips. The plant material needs to be mixed together in equal parts whilst ensuring that the total amount of material is as detailed above. I've used the strips to test 3 plants at once with good results. Of course, a positive test outcome wouldn't show which of the 3 plants is infected. However, depending on the circumstances of the test, there is a statistically optimal number of plants to test at once to reduce the total number of tests needed. Intuitively, if we expect the rate of infection to be low, then testing plants together makes sense.

For example, if a retail nursery is using the strips to batch test a large purchase and expects a low probability of infection, say 1 in 30, then testing 3 plants at once will reduce the initial number of tests from 30 per 30 plants to 10 per 30 plants. If, as expected, on average just one of the tests per 30 is positive, then an average of 2.85 more individual tests must be done on the 3 plants testing positive for an accurate estimation of the total infection rate. This reduces the total number of tests from 30 to 12.85 per 30 plants.

On the other hand, if a collector is using the strips to test plants which are showing visual signs of infection and it turns out that 50% of them are infected, then testing 3 plants together would be marginally less efficient than testing them individually.

To illustrate this, using the notation 'I' for an infected plant, 'C' for a clean plant, 'P' for a positive test and 'N' for a negative test, we can produce a table of possible infection combinations of the 3 plants (Fig. 8). Each combination is equally likely because each plant has a 50% chance of being infected. In each case, we can also calculate the number of subsequent individual tests needed to determine whether each plant is infected. Note that in the case where the first test (using all 3 plants together) is negative, then no further tests are required. By using this method for a 50% infected sample, we would need to perform an average of 3.5 tests per 3 plants.

Fig 8 : *Test table*

Plants	1st Test	Subsequent number of tests required
I I I	P	3
I C C	P	3
I I C	P	3
I C I	P	3
C I I	P	3
C C C	N	1
C I C	P	3
C C I	P	2

Conclusion

We should all be more aware of HVX and how to test for it. Testing is simple, reliable, and easy to interpret. It's based on accepted and validated scientific methods and need not be expensive. Whilst it shouldn't replace good handling practices for hostas (such as disinfecting tools, visual observation of plants, buying from reliable sources etc.), testing must non-the-less play a very important role in the battle against HVX. Finally, with the help and encouragement of the BHHS (possibly including an accreditation scheme), we should expect our suppliers in particular to rise to the challenge and embrace the testing regime; retail hosta buyers have every right to expect that specialist nurseries are regularly testing their stock for HVX.

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