

Help is on the way — don't give up on box just yet

Scientists combining AI with traditional gene mapping are getting closer to adding to the range of buxus with strong resistance to box blight. “There’s no reason to look for alternatives,” says Research Geneticist Dr Fred Gouker from the United States. “We are looking at breeding alternatives so box can retain its status and stature in the landscape,” he adds.

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Popular and extremely important in American gardens and landscapes since its introduction in the 1600s, the box has been a traditional choice for topiary and hedging in many historic and private gardens in Europe because it is resistant to deer.

THE ONGOING HUNT FOR ELITE BOX SELECTIONS

Since the discovery of box blight in the USA in 2011, finding resistant cultivars has been a priority. Cultivars in the NewGen and Better Buxus ranges are now available, but Fred Gouker, who works at the USDA Agri Service based at the National Arboretum in Washington DC, continues to hunt for elite box selections to widen the choice of strong pest and disease resistance, architectural shapes, heat and cold tolerance and foliage colours to improve the overall health of the species and its sustainability within the landscape. This is part of his role in making genetic improvements in woody plants using molecular and traditional applied breeding.

NATIONAL COLLECTION OF BOXWOOD

Within the arboretum grows a national collection of boxwood, which, along with a nursery, is forming the basis of the breeding

programme, just one of a few in the United States. These plants provide not only industry standards but also germ plasm from the 276 accessions, enabling the research of molecular marking for disease detection and disease resistance. These include 184 unique taxa and 68 added since 2012 from the wild and new industry introductions, and plants with distinct characteristics such as *B. wallichiana*, which can reach 6ft and the variegated *B. microphylla* var. *japonica* ‘Kinsha’.

DNA FINGERPRINTING

DNA fingerprinting has allowed plants to be identified on a genetic level, which has led to understanding how they relate to each other. Gouker says this helps inform decisions on what to use for breeding. “If they are too distantly related, we might not even be able

to get a successful cross or, if we really want to find sources of resistance, then we need to move further away from the *Buxus sempervirens* gene pool to improve our chances of getting resistant cultivars or taxa. Knowing this relationship can really help our efforts.”

155 CROSS AND SELF-POLLINATION CROSSES

Running parallel are two programmes. One is for breeding elite populations using traditional breeding methods and stringent selection and testing based on the information gained through DNA fingerprinting. This is a long, slow process taking 10–12 years. The other programme is for genetic mapping populations with the challenge of getting enough progeny to get sufficient material and data. Unusually, the best

(Left) Fred Gouker. (Right) Within the arboretum grows a national collection of boxwood, which, along with a nursery, is forming the basis of the breeding programme, just one of a few in the United States.





results come from crossing two parents with variable disease-resistant characteristics, i.e., an elite cultivar with one that is blight-resistant.

So far, there have been 155 cross and self-pollination crosses, using 23 species as seed parents and 26 as pollen parents, resulting in more than 1,100 germinated seedlings. A selection of 15 F1 hybrids with good horticultural and disease-resistant characteristics have been identified, and cuttings have been distributed for trials within the industry.

SCREENING FOR DISEASE AND RESISTENCE

Currently, only one of the two known boxwood blight pathogens has been identified in the USA, and as it has been found in the national collection, this section is currently closed off to the public. Screening for disease and resistance to find out what is tolerable and what is susceptible is ongoing using leaf assays rather than whole plants, which saves time, effort, and space. After five days from inoculation, using *B. sempervirens* 'Suffruticosa' as a positive control, lesions are measured manually and

through image analysis software. So far, more than 2,382 replicate leaves from 387 F1 progeny plants have been screened, and results have shown that crosses using *B. harlandii* and *B. microphylla* show strong resistance.

AI APPROACHES

This is a long-winded and often quite boring process, so Fred Gouker and his team have started to use AI approaches in the form of Convolutional Neural Networks (CNN) to automatically collect a few images to help in the measuring and scoring of lesions. Using masses of data, this can then classify if the leaf is diseased or healthy more quickly and takes out the subjectivity or bias. This data set is added too with thousands of augmented images of healthy, blighted or leaves with other visual problems such as stress to enable it to be a more accurate approach. Image variations include leaves horizontally and vertically at different rotations and variations of colour, contrast, and saturation. So far, this has helped the CNN programme to be around 98 per cent accurate.

Box is a traditional choice for topiary and hedging in many historic and private gardens. Pictured are the gardens at Château de Villandry, where a range of BetterBuxus was planted.

STANDARDISING THE CLASSIFICATION OF DISEASE SYMPTOMS

To take this further, the team has challenged the software with images including branches, multiple leaves, and natural backgrounds. A new custom CNN model which uses heat map extraction is now being used. The idea is that it will help standardise the classification of disease symptoms, be a more robust and efficient way for breeders to screen material, and be adapted and trained for other plant types. Additionally, the genome of *Buxus sempervirens* has also been identified, and along with another laboratory process, this is also helping to predict and understand the genetics of boxwood and identify susceptibility to box blight.

This article was produced from a webinar: 'In the Lab and Landscape at the National Arboretum: Boxwood Genomics and AI Solutions for Blight Detection and Protection. Hosted by Horticultural Research Institute.