

Host susceptibility of various *Buxus* spp. to box moth (*Cydalima perspectalis* Walker)

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1 | INTRODUCTION

Various observations in gardens and in the nursery have shown that there is a difference in susceptibility to infestation by box moth between the different species and cultivars of *Buxus*. This manifests itself in a different damage pattern at the same pest pressure. *Buxus sempervirens*, our native European species, appears to be very sensitive to damage caused by the caterpillars of boxwood moth, a pest of East Asian origin. *Buxus sempervirens* does not naturally occur in the area of origin of box moth, while Asian species appear to have a higher tolerance to damage. This is probably due to a co-evolution between these *Buxus* species and the boxwood moth. A number of cultivars, originating from these wild species, may also have better resistance. Also our new resistant *Buxus* hybrids (Betterbuxus®) originate from Asian parents and, based on field observations and preliminary tests carried out in April and August 2019, may have a higher tolerance against damage caused by the boxwood moth. The aim of this new trial is to investigate how high this tolerance is in different *Buxus* species in analogy with the *Calonectria* study. (1)

2 | GREAT GENETIC DIVERSITY WITHIN THE GENUS BUXUS

In European gardens mainly one species of boxwood is used, *Buxus sempervirens*. This species is found in nature in large parts of Europe, but only on calcareous soils. (ref. botanical research www.herplant.be).

There is another European species, *Buxus balearica*, which is native to the Mediterranean.

Buxus colchica and *Buxus hyrcana* are similar to *Buxus sempervirens* and also differ very little genetically so that they can be classified genetically better under *Buxus sempervirens*.

In Asia there are about 30 wild species of *Buxus*, most of which are tropical.

The temperate species are hardly cultivated, mainly cultivars of *Buxus microphylla* can be found in gardens and nurseries. The *microphylla* group is a very diverse group and can better be divided into 4 separate genetic groups, according to the most recent research. In this research, we have mainly been working with these temperate species from Asia and our new hybrids.

In the trials of 2019 we worked with 17 different genotypes. In the current trial 13 representative genotypes were chosen, based on previous genetic research of the genus *Buxus* (see Table1). The cultivars *B. Babylon Beauty*, *B. Heritage*, *B. Renaissance* and *B. Skylight* are also known under the brand name Betterbuxus® and are resistant to *Calonectria pseudonaviculata*. (2)

(1) Van Laere K., Heungens K., Gehesquière B., Leus L., Hermans D., and Van Huylenbroeck J., (2019) Breeding and selection of *Buxus* for resistance to *Calonectria pseudonaviculata*. *J Phytopathol*, 1-8.

(2) Van Laere K., Hermans D., Leus L., Van Huylenbroeck J., Genetic relationships in European and Asiatic *Buxus* species based on AFLP markers, genome sizes and chromosome numbers, 2011.

Table 1

Selected Buxus Genotypes

Name	Origin
<i>Buxus Bodinierii</i>	wild collected (China)
<i>Buxus harlandii</i>	wild collected (China)
<i>Buxus henryi</i>	wild collected (China)
<i>Buxus macowanii</i>	wild collected (South Afrika)
<i>Buxus microphylla</i> 'Rococo'	breded
<i>Buxus microphylla</i> var. <i>japonica</i>	wild collected (Japan)
<i>Buxus microphylla</i> var. <i>koreana</i>	wild collected (Korea)
<i>Buxus sempervirens</i>	breded
<i>Buxus wallichiana</i>	wild collected (Nepal)
<i>Buxus</i> 'Babylon Beauty'	hybrid
<i>Buxus</i> 'Heritage'	hybrid
<i>Buxus</i> 'Renaissance'	hybrid
<i>Buxus</i> 'Skylight'	hybrid

3 | BOX MOTH

Box moth (*Cydalima perspectalis*) is a species from the grass moth family and was first described by Walker in 1859. It is indigenous to East Asia and invasive in Europe, first noted on the border between Switzerland and Germany in 2006. The caterpillars cause severe damage to box plants and can defoliate the plant completely in a mature stage in a very short period of time. The plant looks brown and desolate but will re-grow after about 8 weeks. Repeated infestations lead to the death of the plant.

4 | STRUCTURE OF THE TEST

In previous trials in 2019 two or three year old plants in pots were used. In this trial design it was difficult to apply a clear scoring because of the large number of leaves.

Based on a small preliminary study by Hanne Denaeghel (Proefcentrum voor Sierteelt-PCS) and advice from Jochem Bonte (Institute for Agricultural, Fisheries and Food Research -ILVO) the following trial design was chosen. Both researchers also participated in the follow-up of the trial.



Per research object a twig with 20 leaves was used, on which two caterpillars were placed. To reduce the margin of error 10 repetitions were carried out. For the rare buxus species the tests were repeated twice.

A total of 13 genotypes were used, representative of the genetic variation of the moderate European and Asian species: see Table 1.

The plants had not been treated with plant protection products.

The experiment was carried out in a greenhouse to keep away external predators (birds and wasps).

Live caterpillars, with an average length of 12 to 16 mm with a maximum of 25 mm, were collected on untreated plants. The remaining caterpillars were kept in a storage bin and fed daily with fresh twigs of *Buxus sempervirens* to monitor their vitality in captivity. Two caterpillars were released per object (twig) on 6 April 2020. Every day the twigs were checked and kept in optimal condition. The average day temperature was approx. 25°C, the average night temperature 20°C.

On April 10th, April 14th and April 20th 2020 the trial was evaluated: the number of caterpillars or pupae was counted and the vitality of the caterpillars was checked. We monitored infestation on young leaves as well as on old leaves separately.

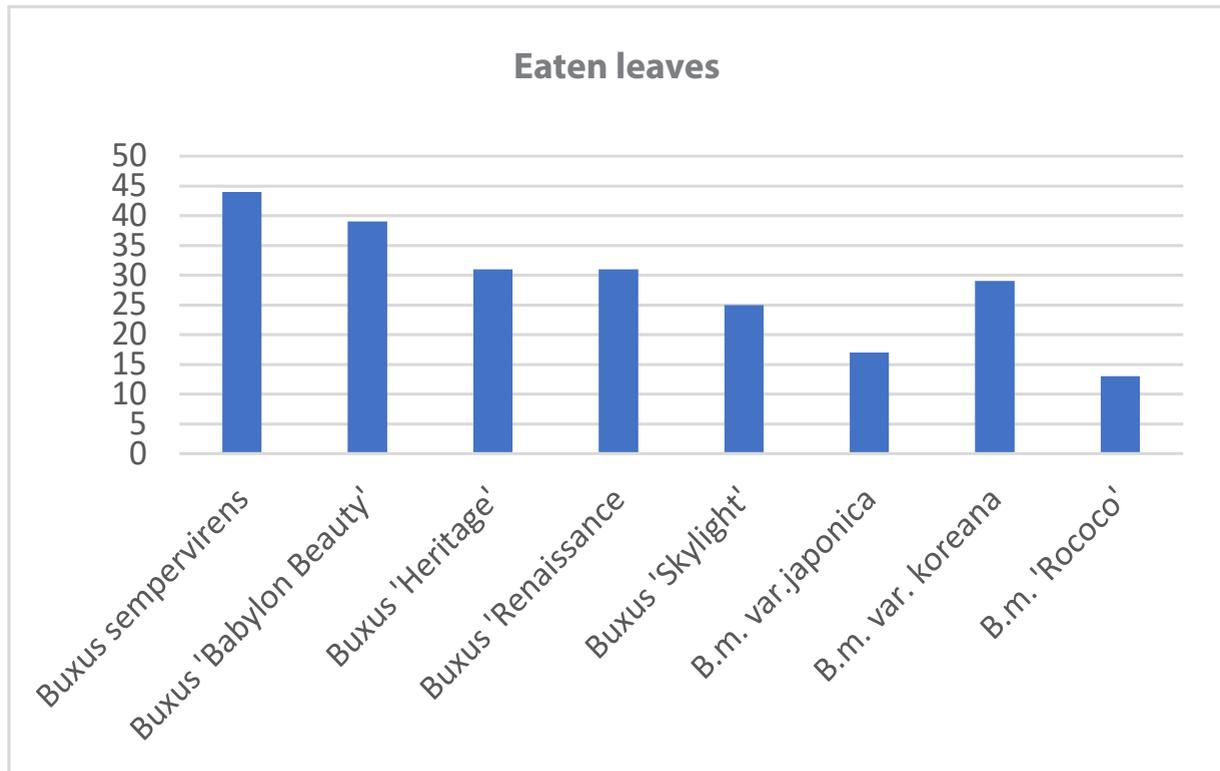


5 | EVALUATION

Most caterpillars started to eat immediately due to the rather high temperature. Soon it became clear that not every species of *Buxus* were equally popular.

5.1 | Number of eaten leaves

Nearly all objects were found to have damage by the caterpillars but there was a large difference in the amount of damage. The exact number of eaten leaves was counted. The graph below shows the total eaten leaves per *buxus* species. *Buxus sempervirens* was affected the worst, with 44 eaten leaves out of 200, *Buxus microphylla* "Rococo" the least with only 13 leaves out of 200. In our new Betterbuxus® hybrids mainly young leaves were eaten and the score varied between 25 and 39/200.

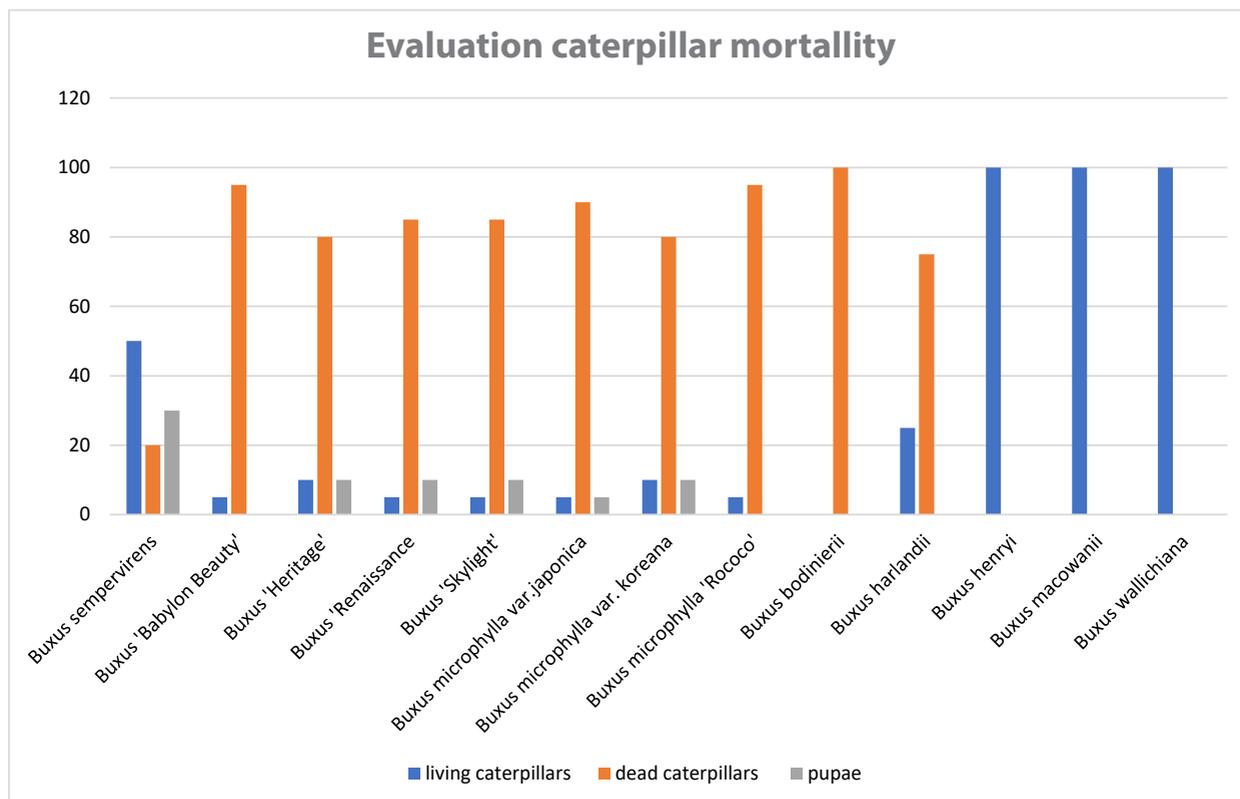


5.2 | Number of living caterpillars and pupae

On April 20th the number of surviving caterpillars and pupae was counted per object. You can see an overview of this in the graph on caterpillar mortality. For the sake of clarity, both tests with 10 repetitions (test A) and the rare species with 2 repetitions (test B) were put in one graph based on percentages on the total amount of caterpillars used per *buxus* species.

In *Buxus sempervirens* the highest number of living caterpillars and pupae was counted after 15 days. All other *Buxus* species in test A had a remarkably smaller number of living caterpillars and pupae than *Buxus sempervirens*. These *Buxus* species showed a negative effect on the development of the caterpillars of the box moth.

In test B we see a similar result for *B. sempervirens*, *B. henryi*, *B. macowani* and *B. wallichiana* in the number of surviving caterpillars. Only in *B. sempervirens* also pupae were found. The development of the caterpillars on *Buxus henryi*, *B. macowani* and *B. wallichiana* may have been a bit slower due to a less suitable food source. However, in the species *B. harlandii* and certainly in *B. bodinierii* more dead caterpillars were found. These *Buxus* species showed a negative effect on the development of the caterpillars of the box moth.



6 | Conclusion

The caterpillars appear to have a great fondness for *Buxus sempervirens*, this was apparent both from the number of leaves eaten and the number of living caterpillars and pupae that were found. This confirms the observations of the damage in our gardens.

Field observations showed that Asian species were less sensitive and sometimes hardly affected by the caterpillars of the boxwood moth. This was largely confirmed in this trial. Some species showed hardly any damage and remained quite green with as outliers the originally Japanese *Buxus microphylla* 'Rococo' and *Buxus microphylla* var. japonica. *Buxus microphylla* var. koreana from Korea and *Buxus microphylla* var. sinica from China appear to be more susceptible to damage. On all these species, however, few living caterpillars and pupae were found. This may play a role in lowering the pest pressure of the box moth in general.

Our new Betterbuxus® hybrids show a high degree of tolerance, with mainly damage to the young leaf. It was found that hardly any pupae were formed and that most of the caterpillars died. This is a nice side effect of our breeding against *Calonectria pseudonaviculata*. Extensive field tests will show whether this conclusion is correct.

The general conclusion from all trials of 2019 and 2020 is that both existing Asian species and the Betterbuxus® hybrids will not be completely eaten bare and will remain green in contrast to the common *Buxus sempervirens*. These species also reduce the pest pressure of the boxwood moth due to a higher mortality and by slowing their development.