Developing Verticillium Resistant Rootstock for Norway Maple

J. A. HIEMSTRA

Plant Research International, 6700 AA Wageningen, The Netherlands

Tel.: +31 317 477 326, Fax: +31 317 418 094, E-mail: j.a.hiemstra@plant.wag-ur.nl

Abstract

Verticillium wilt causes serious losses in the cultivation of shade trees, especially Norway maple (*Acer platanoides* L.). In 1993 research was started aiming at the development of Verticillium resistant rootstocks for Norway maple. Efficient methods to select and screen for resistance in maple were developed, leading to several large-scale selection experiments carried out in the period 1994–1996. This resulted in a first selection of about 300 plants out of a total of nearly 20 000 seedling plants. An attempt was made to propagate these plants vegetatively in order to develop small clones for resistance testing. During this stage of the research many plants were lost because of failure to propagate. After a second screening for resistance 35 plants were selected for further examination. Vegetative propagation was continued and from 2000 on the performance of about 15 clones could be tested on naturally infested fields as well as after inoculation. The results of the field test show a clear selection response with disease incidence in plants of the selected clones being 50% less than in the seedlings and the randomly chosen clones. Within the group of selected clones there was a substantial variation between the clones with some clones having no disease at all whereas in other clones some plants showed serious symptoms. Results of the field tests will be presented and the possibilities for solving the Verticillium wilt problem in maple by means of using clonal root-stocks that are resistant to Verticillium wilt will be discussed.

Keywords: *Verticillium dahliae*; *Acer platanoides*; resistance; selection; rootstock; shade trees

INTRODUCTION

Verticillium wilts in trees almost invariably are caused by the soil-borne fungus *Verticillium dahliae* Kleb. This fungus has a very broad host range including many tree species. Because common field crops such as potato and cotton are also among its hosts, the fungus is widely spread in agricultural soils. In the past decades the Dutch tree breeding industry has strongly expanded, often on former agricultural fields. As a result problems caused by Verticillium wilt are common. Many tree species, including *Acer, Catalpa, Fraxinus, Robinia, Tilia, Ulmus* and *Syringa* species may be affected (HIEMSTRA & HARRIS 1998). In an inventory in 2000 the total annual losses in the Dutch shade tree industry as a result of Verticillium were estimated to amount up to 4.5 million € (HIEMSTRA unpubl. data).

Norway maple (*Acer platanoides* L.) is the most important species in Dutch tree breeding industry that is affected. This very susceptible species is grown mainly as a shade tree through budding of cultivars onto seedling rootstocks. Many different cultivars are grown and all present cultivars as well the seedling rootstocks are susceptible to Verticillium wilt. Because natural infection in trees takes place through the root system, development of a resistant rootstock would solve the Verticillium problem in Norway maple. With this aim a research project was started in 1993. This paper summarises 10 years of research, including development of methods to select and screen for Verticillium resistance in trees, large scale selection, propagation of selected material, and finally field testing of the resulting new clones.

Methods for selection and screening

In a first screening of the existing cultivars no resistance at all was found. Therefore it was decided to search for resistance in seedling populations of Norway maple. Many different methods varying in the

way of inoculating plants (through soil, root or stem), inoculum level and type of inoculum were tested and compared in a search for methods that on the one hand could deal with many plants in little time and on the other hand were as effective in getting plants infected as possible. Two methods were developed for use in large-scale selection experiments. Firstly, in a field experiment one year old seedling plants were stem-inoculated by cutting into the xylem through a drop of a conidial suspension, in a way very similar as this is practised in Dutch elm disease research. Secondly, in order to be able to handle large numbers of trees, in the greenhouse very young seedlings (4–6 weeks after germination) were inoculated by dipping their bare roots into a conidial suspension of *V. dahliae* (1×10^6 conidia/ml).

Large scale selection

In a field experiment 2000 one-year-old plants, grown from four different seed provenance's were stem-inoculated. Disease development in individual trees was regularly rated in the season of inoculation. Early in the next growing season trees were checked for dieback and overwinter death and regrowth after the dormant season was rated. The best 55 plants were selected and vegetative propagation by means of rooting of cuttings and budding onto seedling rootstocks was started.

In addition in the greenhouse a large-scale selection experiment was carried out. About 17 500 seedlings were grown from seed from 9 different provenances. All plants were root-dipped as described above in a series of consecutive experiments. Plants developing symptoms of wilt were discarded weekly untill about 4 months after inoculation. At that time vegetative propagation through rooting of cuttings of the remaining 260 plants was started.

Although many clones were lost and the remaining clones often consisted of only 2–5 plants, a first test of the selected material was possible. Rooted cuttings as well as plant material budded onto seedling rootstocks was stem-inoculeted and symptom development was recorded regularly. The results showed a very clear selection response with disease levels in selected material on average being much lower than in non-selected material (Figure 1).

Vegetative propagation

Rooting of Norway maple cuttings is notoriously difficult. Therefore CPRO-DLO (now Plant Research International) and the plant tissue culture laboratory of the Applied Plant Research (COWT-Lisse) started research into developing methods to propagate Norway maple through tissue culture Protocols were developed that allowed *in vitro* propagation of part of the selections. However, after several years the research was ended because the propagation ratio was still rather low and not all selections responded well. In addition the Dutch Applied Plant Research (PPO-Boskoop) started work on improving methods for rooting of maple cuttings. Although not all selections responded well, finally methods were developed

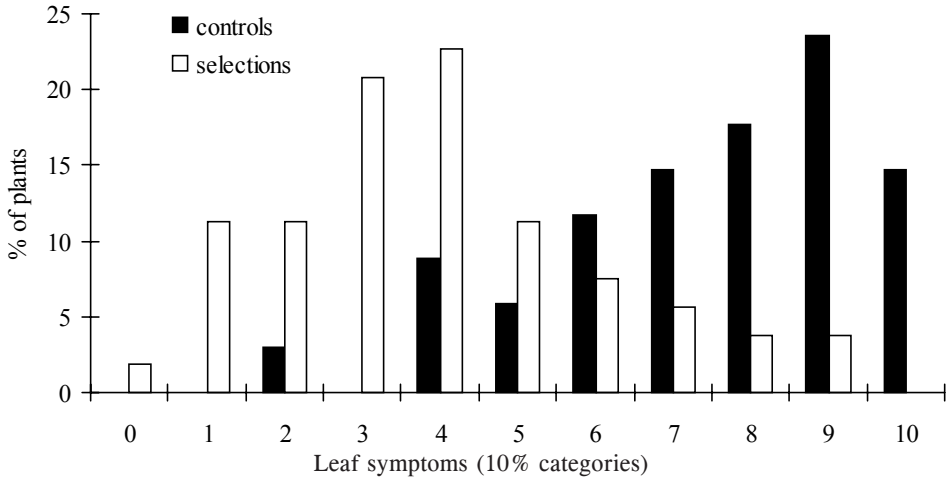

Figure 1. Results of testing selected material and non-selected seedling plants for resistance to *Verticillium dahliae*. For each of the two groups the distribution over the disease categories (10% categories; i.e. from no symptom on left to 100% symptoms on the right of the *x*-axis) is given

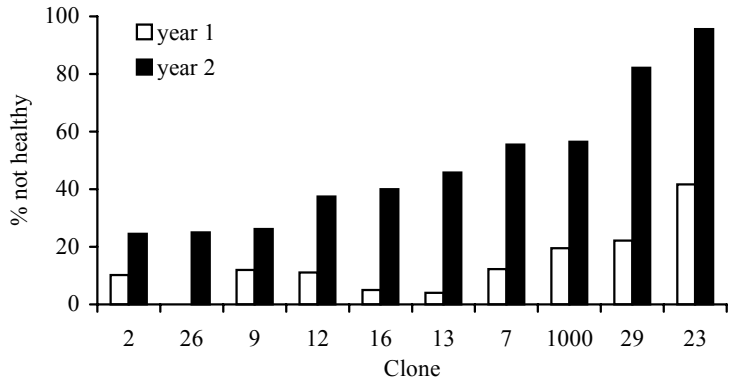

Figure 2. Results of testing individual selections on an heavily infested field (30–55 mscl/g soil). For each of the clones the percentage of diseased trees is given; number 1000 is the control group (seedlings) and numbers 29 and 23 are two clones that were known to be very susceptible (negative controls)

that allowed large-scale propagation of many other selections. These methods were used in an attempt to develop the 35 best performing selections (i.e. ease of propagation and good results in the first resistance tests mentioned above) into clones for field testing.

Field testing of selected material

From the year 2000 onwards the resistance level of about 15 selections is being tested on a naturally infected field. Each year thirty to fifty rooted cuttings from several selections are planted in a randomised block design on a field with an inoculum density of 30–55 microsclerotia per gram of soil. Disease symptoms are recorded several times during the growing season. The results so far are very promising. The best selections apparently are not completely resistant, but on heavily infested fields the disease incidence in these selections is much lower than in non-selected plants (Figure 2). Because the soil inoculum level is extremely high this suggests that under more normal conditions the resistance of the new selections may be very satisfactory. Tests comparing these selections with recent selections from the UK (HRI-East Malling) and with the cultivars Parkway and Jade Glen, that in the USA are considered to be resistant (TOWNSEND *et al*. 1990), were started in 2002.

DISCUSSION AND CONCLUSIONS

Maples are among the most susceptible tree hosts of V. dahliae (Hiemstra & Harris 1998) but some resistance in seedlings has been reported in A. rubrum (Townsend & Hock 1973) and also in A. platanoides (Valentine et al. 1981). The present data confirm that within seedling populations of A. platanoides variation in susceptibility to V. dahliae does exist. They also show that it is possible to select for resistance; effective methods to select and screen for resistance have been developed, and the resulting clones perform much better on seriously infected fields than non-selected seedlings or standard clones do.

References

HIEMSTRA J.A., HARRIS D.C. (eds) (1998): A compendium of Verticillium wilt in tree species. CPRO-DLO/HRI, Wageningen/East Malling.

TOWNSEND A.M., HOCK W.K. (1973): Tolerance of half-sib families of red maple to Verticillium wilt. Phytopathology, **63**: 673–676.

TOWNSEND A.M., SCHREIBER L.R., HALL T.J., BENTZ S.E. (1990): Variation in response of Norway maple cultivars to *Verticillium dahliae*. Plant Dis., **74**: 44–46.

VALENTINE F.A., CARLSON K.D., WESTFALL R.D., MANION P.D. (1981): Testing Verticillium wilt resistance in urban Norway maples. J. Arboricult., **7**: 317–325.