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Twenty-four years of Dutch Trig® application to control Dutch elm disease

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Abstract Since 1992 elm trees have been treated with a biological control product Dutch Trig® to protect them from infection by *Ophiostoma novo-ulmi* causing Dutch elm disease (DED). The active ingredient of the biocontrol product consists of the fungus *Verticillium albo-atrum* strain WCS850. A conidiospore suspension of this fungus is injected into the vascular system of elm trees at a height of 1.3 m. This biocontrol product prevents healthy elm trees from fungal infection transmitted by elm bark beetles. Dutch Trig®, however, does not protect already infected trees or trees connected with diseased trees via root grafts. Since 2010, only 0.1 % of the injected elms became infected with DED through beetle transmission and an additional 0.4 % of the treated elms were infected through root contact in the Netherlands. Regression analysis considering all injected elm trees in the Netherlands since 1992 indicated that DED infection through beetle transmission had significantly decreased during the 24 years application of Dutch Trig®. In 2015, approximately 28,300 trees in five countries (Netherlands, USA, Germany, Canada and Sweden) were treated with Dutch Trig® to protect valuable susceptible elm trees, mainly in urban environments.

Keywords Biological control agent · Commercial application · Induced resistance · *Verticillium albo-atrum*

Introduction

Elms are important trees along roads and canals, as well as in coastal areas, due to their resistance to harsh conditions such as (sea) wind, de-icing salts and their very good recovery capacity from mechanical damage. The fact that elms grow in most soil types endure narrow root space and closed pavements make them an ideal tree for urban environments (Buiteveld et al. 2015). Several elm varieties used in cities are appreciated for their attractive architecture and their light canopy giving shelter on hot days but without complete blocking of sun light. Elms are also of great ecological value being the habitat for numerous insects, butterflies and lichens that sometimes specifically occur on elm trees (Hiemstra et al. 2006).

Unfortunately, a devastating disease, the so-called Dutch elm disease (DED), appeared in Europe during the 1920s and in North America by 1930. Sudden wilting and dying of the leaves and branches was caused by the fungus *Ophiostoma ulmi* (Buisman) Nannfeldt during the first pandemic from 1920s to

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1940s. The spreading of a new, even more aggressive species *O. novo-ulmi* Brasier after 1972 is responsible for the current pandemic. This species also infected large numbers of newly planted trees that were supposed to be less susceptible to DED. Since 1928, DED has killed tens of thousands of elms annually in Europe and North America (Buchel and Cornelissen 2000).

DED is transmitted by bark beetles mainly belonging to the genus *Scolytus*, that reproduce in weakened and dead elms. If these trees are infected with *Ophiostoma* spp., beetles emerging from the trees transport conidia of the fungus to healthy elms during their feeding activity on elm twigs (Webber and Brasier 1984). Subsequently the fungus develops in the xylem, and the infected weakened tree becomes suitable for breeding of the beetles in the stem bark. Most common species spreading the pathogen worldwide are *Scolytus scolytus* and *S. multistriatus* (Santini and Faccoli 2015), whereas *Hylurgopinus rufipes* is an important vector in areas with cold winters (Buchel and Cornelissen 2000). A second infection path is through root contact between infected and healthy trees, since the fungus is able to move from one elm tree to another through grafted roots (Buchel and Cornelissen 2000).

Different methods to control DED have been applied with more or less success since the first outbreaks of DED. Strategies included reducing the beetle populations chemically, biologically or by pheromone trapping, injecting trees with fungicides, application of antagonistic micro-organisms, removal of DED-killed elm trees, and selecting resistant elm varieties (Buchel and Cornelissen 2000; Díaz et al. 2013). In the Netherlands a national sanitation program, regulated by law between 1977 and 1991, reduced the loss of elms to an average of 1 % per year. However, after 1991 when this law was abolished the annual number of new trees being affected increased again up to 10–15 % trees (Hiemstra et al. 2006). In some Dutch areas strict sanitation is still successfully applied, e.g. in Amsterdam the annual infection incidence was kept at a level of 0.4–1 % (Bleeker and Kaljee 2013, 2014). In the Dutch province Friesland a coordinated sanitation program started in 2005, reducing the infection percentages from 8 % in 2005 to 1 % since 2009 (Anonymous 2015). These low percentages are to some extend positively influenced by the presence of resistant elm varieties, as well as by the treatment of a number of valuable trees with the biocontrol product Dutch Trig® (Bleeker and Kaljee 2014). The efficacy of monitoring and strict sanitation was also recently evaluated in Canada, showing that rapid removal of infected trees, i.e. 79 % removal by 1 October, reduced the infection rate to 1.5 % compared to 3.1 % when affected trees were removed in autumn or winter (Veilleux et al. 2012). However, such a rapid removal is a practical challenge.

Nowadays, elm cultivars with a high level of resistance to DED are available on the market (Buiteveld et al. 2015; Martín et al. 2015b). This is an important solution for the use of elm trees in the future, but it does not protect the existing valuable trees from infections.

A successful method to protect existing susceptible elms against infection with DED is to inject trees with the biological control product Dutch Trig® (Scheffer et al. 2008). Elms are injected once every year in spring, as soon as the leaves have started to sprout after flowering of the elm and preferably before the beetle infects trees with DED. The active ingredient of this product is the fungus *Verticillium albo-atrum* WCS850, which enhances the natural defence mechanism of elms (Buchel and Cornelissen 2000; Scheffer 1990). Conidiospores of this fungus that are injected in the xylem tissue induce resistance when germinating in the tree. The fungus survives during the season but remain close to the site of inoculation (Scheffer 1990). This biocontrol agent has been commercially applied in the Netherlands since 1992, and is subsequently registered and used in several other countries (Table 1).

The efficacy of the product in controlling DED has been demonstrated in field trials with artificial inoculated elms before registering the product in the Netherlands (Buchel and Cornelissen 2000; Elgersma et al. 1993; Scheffer 1990) as well as in USA (Scheffer et al. 2008). However, the efficacy of the biocontrol agent as commercial product under practical conditions with natural pathogen infections, as well as the experience with the application procedure are crucial for the success of a product. In this article we therefore focus on the large-scale application, i.e. the results of 24 years application of Dutch Trig® as a commercial product are presented. The efficacy of the treatment was compared between the successive years. Since this product is used to protect valuable trees, untreated

Table 1 Dutch Trig® application and registration in different countries

	Number of treated trees in 2015	First experimental introduction	Year of registration
The Netherlands	21,500	1992	1992
USA	3000	1995	2005
Germany	300	2006	2008
Canada	2000	2008	2009
Sweden	1500	2008	2010

control trees were not included in the commercial injection schedule. Only in few locations, DED incidence in treated as well as untreated elm trees were available.

Materials and methods

The biocontrol agent and its production

The active ingredient of Dutch Trig® consists of conidiospores of *Verticillium albo-atrum* WCS850 (previously identified as *V. dahlia*). This strain is a naturally hyaline form of *V. albo-atrum* which is not producing resting structures. Conidiospores were produced under sterile conditions on Czapec Dox agar and harvested in distilled water, obtaining a suspension with 1.0×10^7 spores ml^{-1}. Spore concentration, colony formation and absence of impurities and contaminants of each batch were checked. Spores were stored in 40 ml vials at 4 °C during maximally seven weeks.

Method and timing of application

Trees were injected (vaccinated) with Dutch Trig® once a year in spring, as soon as the leaves had started to sprout after flowering of the elm. The best timing of the injections is at 25 % leaf expansion. For the Netherlands as well as North America vaccination usually started the first or second week of May and the second week of June vaccinations were finished. The vaccine was injected into the tree trunk at a height of 1.3 m, every 10 cm of tree circumference, by pushing the chisel through the bark and releasing one drop (0.15 ml) with one pull of the trigger (Fig. 1). The injection system prevents spills and any impact on plants, trees or animals in the environment. The method is fast, on average 2–3 min per treated tree, which enables treatment of large numbers of trees in a short time frame.

Fig. 1 Hand held tree injection system: the chisel is pushed through the bark, by pulling the trigger a drop of the vaccine is released which subsequently will be taken up by the elm trunk (photo by BTL Bomendienst)

Product registration and commercial application

For commercial application, the biocontrol agent was registered in the respective countries. The product Dutch Trig® is registered in the Netherlands since 1992 (Table 1). After extensive testing in the USA since 1995, full registration was achieved at the Environmental Protection Agency (EPA) in 2005 (http://iaspub.epa.gov/apex/pesticides/f?p=PPLS:8:10808689629105::NO::P8_PUID:393416). Since 2008, the active ingredient has been approved on the Annex I list of the EU Directive 91/414/EEC (http://ec.europa.eu/sanco_pesticides/public/?event=homepage). More recently the product was registered in Germany, Sweden, and Canada (Table 1) and a registration in the UK is currently in process. In 2015, the total number of treated elms in the five countries was approximately 28,300 (Table 1).

Monitoring DED symptoms

All injected trees in the Netherlands were checked for DED symptoms twice in the year of treatment, i.e. in week 27 and 37. These observations were collected in a questionnaire by BTL Bomendienst (Apeldoorn, the Netherlands). In an early stage of infection, DED symptoms appear as yellow/brown dried out leaves at the ends of branches which stay on the branch tip, forming small "flags". In a more advanced stage, whole branches in the crown dry out and then in the final stage the whole crown of the tree becomes yellow, brown and finally bare. Also internal symptoms, i.e. vascular discoloration, were checked. A distinction was made between (1) *Ophiostoma* infection starting with one or few branches in the tree canopy and spreading downwards, which was considered to be due to beetle transmission, and (2) infection starting in the root system spreading through the whole tree, which was considered to be transmitted through root grafts with adjacent already infected trees. DED symptoms were also evaluated at a few locations with untreated elms.

Experimental design

The study consists of the evaluation of the incidence of DED in susceptible elm trees following application of Dutch Trig® on a commercial basis. A total of 573,018 trees were treated with Dutch Trig® in the Netherlands during a period of 24 years. In order to protect valuable elm trees, no general untreated controls were considered. Instead, untreated elms were evaluated in comparison with treated ones at three locations.

Statistical analysis

Statistical analysis were performed with the program GenStat 17th Edition (Rothamsted Experimental Station, Harpenden, UK). Since the observations relate to commercial treatments of elms with Dutch Trig®, no replications and randomization of treatments were present. However, data of the different years could be considered as more or less independent observations, since the vaccination protects the elm only during one growing season (Elgersma et al. 1993). Moreover, the injected tree population fluctuated to some extend from year to year. A change in the incidence of infections during the 24 years application of Dutch Trig® was analysed with regression analysis.

Results

DED symptoms in treated trees in the Netherlands

The first years of Dutch Trig® application in the Netherlands, from 1992 up to 1999, the number of annually treated trees increased from 3000 up to 24,000. In this period, approximately 0.6 % of injected trees became infected with DED through beetle transmission, and a similar percentage was infected through root grafts or otherwise (Fig. 2). Between 2000 and 2009 around 30,000 elms were treated annually and the infections by beetle transmission ranged from 0.1 to 0.5 %. Since 2010 infection by beetle transmission was 0.1 % or even less. Regression analysis demonstrated that this percentage of infection by beetle transmission after Dutch Trig® application decreased significantly during the 24 years of application with an annual decrease of −0.030 (significant at $P < 0.001$ with t-value = −5.71 and 22 df). Regression analysis resulted in the following formula:

$$Y = 0.722 - 0.030 \times \text{Year of application},$$

where Y = infection % due to beetle transmission after Dutch Trig® treatment, and the year of application starts with 1992 being year 1. The percentage variance accounted for was 57.9 % and the SE of observations was estimated to be 0.180. Infection by root grafts also decreased significantly, but with a smaller annual decrease of −0.013 ($P < 0.004$ with t-value = −3.22 and 22 df) and the percentage variance accounted for was only 29.0 %.

DED symptoms in untreated trees in the Netherlands

A limited number of data concerning infections in untreated elms at similar locations and years as treated elms were available (Table 2). In Amsterdam, with its strict sanitation program, only 0.44 % of the non-treated elms (including trees of resistant varieties) became infected in 2013, whereas all 90 elms that were treated with Dutch Trig® remained healthy

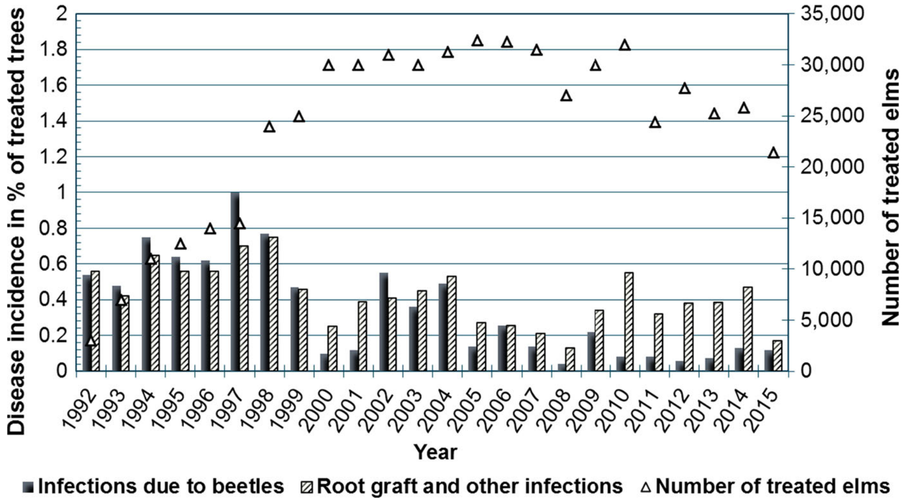

Fig. 2 Number of treated elms and DED incidence among the treated trees caused by beetle infections or root graft and other infections in the Netherlands per year since 1992 (data provided by BTL Bomendienst)

Table 2 Number of untreated and Dutch Trig® injected (treated) elms and DED incidence at different locations in the Netherlands

Location	Period	Untreated elms		Treated elms	
		% infected	Number of elms	% infected	Number of elms
Amsterdam	2013	0.44	75,000[a]	0.00	90
Rotterdam	2007–2013	3.47	647[b]	0.86	3416
Waddinxveen	2014	1.68	179[b]	0.00	132

[a] Including an unknown number of resistant elms

[b] Only susceptible elm varieties

(Bleeker and Kaljee 2014). Also in Rotterdam the infections of treated as well as untreated elms were evaluated during several years, showing lower numbers of DED infections in the treated compared to the untreated elms (Table 2). Another interesting location was Waddinxveen, where two rows of susceptible elms were present close to each other, only separated by a ditch (Fig. 3). Some of the untreated elms were DED infected in 2014, whereas the Dutch Trig® injected trees remained healthy. These examples indicate that Dutch Trig® inoculated trees had fewer DED infections than untreated trees, however too few data were available for statistical analysis (Table 2).

Discussion

Biological control of DED with Dutch Trig® is now applied on a commercial basis during 24 years. The efficacy of this product was proven in several field trials with artificial inoculum of *O. novo-ulmi* reaching high disease levels in the controls (Buchel and Cornelissen 2000; Elgersma et al. 1993; Scheffer 1990; Scheffer et al. 2008). Demonstration of the efficacy of this product under practical conditions with natural DED infections is more complicated. Due to the much lower infection levels with natural occurring inoculum, large numbers of trees should be tested. Moreover, valuable trees cannot be sacrificed as untreated controls.

To demonstrate the efficacy of Dutch Trig® under natural field conditions, results upon infection percentages of untreated elms in comparable locations and years are needed. After the national sanitation program in the Netherlands stopped in 1991, the annual number of trees being affected increased up to 10–15 % trees (Hiemstra et al. 2006), whereas on average 1 % of Dutch Trig® treated elms were infected annually in the period between 1992 and 2004. In the period since 2005 the percentage infection

Fig. 3 Untreated susceptible elms with DED infection (*white cross*) along the provincial road (*left*) and Dutch Trig® treated elms along the street at the other side of the ditch in Waddinxveen (Google street view, August 2014)

in treated trees was even reduced to approximately 0.6 %. Also in areas with a strict and coordinated sanitation program during a prolonged period, low infection levels of 1 % or less can be reached (Anonymous 2015; Bleeker and Kaljee 2013, 2014). However, unknown numbers of resistant elm trees positively influence this infection percentage. In the few locations where Dutch Trig® injected and untreated elms have both been monitored, the infection percentages of the treated trees are always lower, although a statistical significance could not be calculated.

Regression analysis considering all 573,018 elms that were treated during the 24-years period with Dutch Trig®, supported the impression that protection by the biocontrol product had improved during this period. Especially the infection with DED through beetle transmission had decreased and was only 0.1 % of the injected elms since 2010. Several explanations can be given: (i) the continuous effort to optimize product quality and storage conditions, (ii) better practices concerning hygiene and accurateness for the arborists doing the injections, and (iii) environmental factors including reduced disease pressure.

The mode of action of Dutch Trig® is through induction of resistance responses of the elm (Buchel and Cornelissen 2000; Elgersma et al. 1993; Scheffer 1990). Consequently, already infected trees will not be protected by injection with the biocontrol product. Another serious limitation is that the induction of host-defence mechanisms by pre-inoculation does not offer protection against root-graft-mediated DED infection (Elgersma et al. 1993). Thus, once DED has become established in a community of susceptible elms, spread through root grafts cannot be prevented by injections with Dutch Trig®. The practical experience with injecting elms, regularly encountering infected trees in the vicinity of still healthy trees, is supporting this conclusion. Therefore, preventive eradication or elimination of root contact is advised in these situations.

The costs of the injection with Dutch Trig® is depending on the size and number of trees to be treated. In the Netherlands, where approximately 25,000 up to 30,000 trees are treated annually, the price per tree varies between 16 and 25 €. However, the treatment has to be repeated annually since Dutch Trig® gives no protection in the year after its injection (Elgersma et al. 1993). Consequently, only the highly valuable trees, i.e. old and characteristic trees in urban environments, are treated. In these situations the costs of annual injections are affordable since the removal of dead trees, subsequent transplantation, and initial maintenance of new trees are expensive. A published study in Manitoba, Canada, illustrates these costs: 350, 600, and 9600$ for respectively removal of infected trees, replacement of the trees, and loss of aesthetic value and other contributions of boulevard trees in an urban environment (Veilleux et al. 2012).

A practical concern about the product is the fact that living conidiospores are needed for an effective biocontrol of DED since the spores must germinate in the tree to induce the resistance (Scheffer 1990). The survival period of conidiospores is limited. Consequently, shelf-life of the product is limited and storage and transport must be at low temperature to keep the spores alive.

Currently, there is a renewed interest in the development and application of reliable biological control products. Before the development of *Verticillium albo-atrum* WCS850 as a biocontrol product, several *Pseudomonas*, *Verticillium*, as well as non-aggressive *Ophiostoma* strains have been tested to induce the resistance response in elms (Buchel and Cornelissen 2000). In a later study, experiments with *Verticillium dahliae* strain Vd-48 significantly reduced DED symptoms, but the results were not consistent (Solla and Gil 2003). To find a biocontrol strain that is not needed to be inoculated annually, endophytic fungi naturally occurring in the elm xylem were isolated (Díaz et al. 2013). These strains were further selected for in vitro antagonistic activity against the pathogen (Díaz et al. 2013) and competition for nutrients (Blumenstein et al. 2015), but field trials with elm trees with four of these strains showed no repeatable control of DED (Martín et al. 2015a). Selecting an effective, environmentally safe and commercial valuable biocontrol agent requires many crucial choices (Köhl et al. 2011). Costs of production and application of Dutch Trig® are relatively high and the shelf-life is limited. Nevertheless, due to the specialised application by tree arborists, the short period of time suitable for treatment, and the high value of trees, this product became a commercially affordable biocontrol agent, registered in Europe as well as in USA and Canada.

Approximately 100 years after the first introduction of DED in Europe, an effective commercial biocontrol product is available as a preventive treatment of susceptible elm trees against DED transmission by beetles. Injection of trees with conidiospores of the fungus *V. albo-atrum* WCS850, product name Dutch Trig®, reduces the infection by beetle transmission to only 0.1 % of the treated trees. However, the product does not prevent elms from infection through root grafts nor can it cure already infected trees. Consequently, Dutch Trig® is a valuable component of an integrated control strategy to control DED in elm trees. The main elements of such a strategy are acute removal of diseased trees (sanitation), replanting new resistant varieties, and protecting valuable standing susceptible trees through biological control using Dutch Trig®.

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References

Anonymous (2015) Iepenwacht Friesland. Stichting Iepenwacht Friesland. http://www.iepenwachtfryslan.nl/

Bleeker D, Kaljee H (2013) Beheersing boomziekten en plagen in Amsterdam. Jaarverslag 2012. Gemeente Amsterdam, Dienst Ruimtelijke Ordening. https://www.amsterdam.nl/gemeente/organisatie/ruimte-economie/ruimte-duurzaamheid/ruimte-duurzaamheid/publicaties-ruimte/boomziekten-plagen/

Bleeker D, Kaljee H (2014) Beheersing boomziekten en plagen in Amsterdam. Jaarverslag 2013. Gemeente Amsterdam, Dienst Ruimtelijke Ordening. https://www.amsterdam.nl/gemeente/organisatie/ruimte-economie/ruimte-duurzaamheid/ruimte-duurzaamheid/publicaties-ruimte/boomziekten-plagen/

Blumenstein K, Albrectsen BR, Martín JA, Hultberg M, Sieber TN, Helander M, Witzell J (2015) Nutritional niche overlap potentiates the use of endophytes in biocontrol of a tree disease. BioControl 60:655–667

Buchel AS, Cornelissen BJC (2000) Dutch elm disease: an interactive approach. University of Amsterdam & Utopa Foundation. http://www.dutchelmdisease.org/start.html

Buiteveld J, van Der Werf B, Hiemstra JA (2015) Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*. IForest 8:158–164

Díaz G, Córcoles AI, Asencio AD, Torres MP (2013) *In vitro* antagonism of *Trichoderma* and naturally occurring fungi from elms against *Ophiostoma novo-ulmi*. For Pathol 43:51–58

Elgersma DM, Roosien T, Scheffer RJ (1993) Biological control of Dutch elm disease by exploiting resistance in the host. In: Sticklen MB, Sherald JL (eds) Dutch elm disease research. Springer, New York, pp 188–192

Hiemstra JA, Buiteveld J, Kopinga J, Kraneborg KG, Ravesloot MBM, van der Sluis BJ, de Vries SMG (2006) Belang en toekomst van de iep {importance and future of the elm}. PPO-bomen, Lisse, The Netherlands, Report 421

Köhl J, Postma J, Nicot P, Ruocco M, Blum B (2011) Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biol Control 57:1–12

Martín JA, Macaya-Sanz D, Witzell J, Blumenstein K, Gil L (2015a) Strong *in vitro* antagonism by elm xylem endophytes is not accompanied by temporally stable *in planta* protection against a vascular pathogen under field conditions. Eur J Plant Pathol 142:185–196

Martín JA, Macaya-Sanz D, Witzell J, Blumenstein K, Gil L (2015b) Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. IForest 8:172–180

Santini A, Faccoli M (2015) Dutch elm disease and elm bark beetles: a century of association. IForest 8:126–134

Scheffer RJ (1990) Mechanisms involved in biological control of Dutch elm disease. J Phytopathol 130:265–276

Scheffer RJ, Voeten JGWF, Guries RP (2008) Biological control of Dutch elm disease. Plant Dis 92:192–200

Solla A, Gil L (2003) Evaluating *Verticillium dahliae* for biological control of *Ophiostoma novo-ulmi* in *Ulmus minor*. Plant Pathol 52:579–585

Veilleux J, Leferink J, Holliday NJ (2012) Rapid removal of symptomatic trees reduces Dutch elm disease infection rates. Arboric Urb For 38:99–104

Webber JF, Brasier CM (1984) The transmission of Dutch elm disease: a study of the process involved. In: Anderson J, Rayner ADM, Walton D (eds) Invertebrate-microbial interactions. Cambridge University Press, Cambridge, pp 271–306

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