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# VEGETATIVE COMPATIBILITY IN *VERTICILLIUM DAHLIAE* FROM SEVERAL EUROPEAN COUNTRIES[1]

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## Abstract

The vegetative compatibility of 31 *Verticillium dahliae* isolates from Europe is limited. Only two VCGs were detected: VCG 2 (subgroups 2A and 2B) and VCG 4 (subgroups 4A and 4B). However, all isolates included to subgroup 2A or 4A were also compatible to a lesser extent with 2B or 4B testers, respectively. The same applies to the isolates in 2B and 4B subgroups. Three bridge isolates produced differentiated heterokaryons with testers of both main groups. Two isolates were weakly compatible with VCG 3, and strongly with VCG 4 tester. Similarly, one isolate was weakly compatible with VCG 1 and strongly with VCG 2. Six isolates, from Spain and Greece, could not be characterized due to lack of *nit* mutants or reversion of the mutants recovered. Four isolates identified by PCR as *V. longisporum* could not be characterized either as they did not produce *nit* mutants. Altogether out of 25 isolates characterized 15 represented VCG 2 and 10 isolates – VCG 4.

**Key words:** *Verticillium dahliae*, European countries, different host-plants, vegetative compatibility grouping

## Introduction

*Verticillium dahliae* as a wilt disease pathogen is harmful to a broad range of plants. During the last decade isolates of *V. dahliae* were repeatedly characterized on the basis of various parameters, such as morphology (Karapapa et al. 1997), colony growth (Korolev et al. 2000), pigment secretion, sporulation (Zeise and von Tiedemann 2001), vegetative compatibility (Joaquim and Rowe 1990, Chen 1994, Daayf et al. 1995, Elena and Paplomatas 1998, Hiemstra and Rataj-Guranowska

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2003), analysis of DNA (Okoli et al. 1993, Pérez-Artés et al. 2000). However, genetic diversity was studied mainly by means of vegetative compatibility.

In the United States Joaquim and Rowe (1990) identified four VCGs, two of them (VCG 2 and VCG 4) dividing into two subgroups. Further studies, performed in Europe and Japan (Harris and Yang 1995, Daayf et al. 1995, Elena and Paplomatas 1998, Ebihara et al. 1999, Korolev et al. 2000, Hiemstra and Rataj-Guranowska 2003), showed that nowhere all these groups occurred in the same district.

The aim of the present study was to determine the VCGs variability among *V. dahliae* isolates originating from various European countries, from quite different host-plants. The isolates were obtained from main crops and minor crops. In North Europe the main crops were *Acer*, chrysanthemum, potato, rose and strawberry while the minor crops were cherry, hop, catalpa, lilac, barberry. In South Europe the main crops were cotton, olive, tomato and the minor crops were melon, artichoke, sweet pepper, *Xanthium*. Such a careful selection of broader range of host-plants was necessary, as it has been shown recently on the limited number of isolates from trees in The Netherlands that VCGs differentiation was limited to two groups: VCG 2 and VCG 4 (Hiemstra and Rataj-Guranowska 2003). In The Netherlands the *Verticillium* wilt disease is still a serious problem in tree nurseries, mainly for shade trees and ornamental species.

This study was therefore performed on a group of isolates originating from a broad range of host-plants. The project was performed to provide information and tools for elaboration strategies for managing *Verticillium* wilt in trees.

## Materials and methods

### Isolates

Isolates of *V. dahliae* from plants and soil were isolated mostly in 1998–2000, as previously described (Hiemstra 1995, Korolev and Katan 1997, Elena and Paplomatas 1998). Monoconidial cultures were prepared from each isolate and kept on potato dextrose agar (PDA) at 4°C.

31 isolates of *V. dahliae* and four of *V. longisporum*, of different geographical origin, were characterized. *Verticillium dahliae* isolates consisted of black wild-type or hyaline subcultures. The hyaline isolates lost their ability to produce microsclerotia most probably due to many transfers and long maintenance in several laboratories under different conditions.

### Recovery of *nit* mutants

The mutants were recovered according to the method adapted by Puhalla (1985) and modified by Rataj-Guranowska (2001). Agar plugs (5-cm-diameter) overgrown with mycelium were cut from the colony margin and placed at four

points of 9-cm-diameter Petri plates on PDA medium or minimal medium (Correll et al. 1987), containing 1.5 or 6% of potassium chlorate. After 14–21 days of incubation at 27°C small sectors (the putative mutants) taken from the margins of colonies were transferred to basic minimal medium without additives (Correll et al. 1987). The putative mutants were placed at 20 points per 9-cm-diameter plate and incubated at 27°C.

### Characterization of *nit* mutants

During the next 21 days of incubation all the true mutants were transferred to 5-cm-diameter plates as stock subcultures. They were phenotyped on basic medium amended with sodium nitrite (0.5 g/l) or hypoxanthine (0.2 g/l). The original subcultures of mutants were still observed on large plates. There, in random combinations of mutants, starting from the fifth day of incubation, heterokaryon formation *in situ* was observed. That allowed preliminary selection of compatible mutants. At the same time the preliminary selected mutants, usually one to three *nit* 1 mutants, and all the identified *nit* M mutants were used for the next step of work, in which 10–12 mutants were paired in all possible combinations. One or two pairs that appeared most efficient in heterokaryon formation within the isolate were selected for tester-isolates (Rataj-Guranowska 2001).

### Complementation test

All possible combinations between isolates were tested. We tested heterokaryon formation within all the isolates. All tester-isolates were also paired with the international testers of VCGs (Table 1).

## Results

A special effort to generate *nit* mutants yielded differentiated numbers of mutants from each isolate: 4–77 mutants per isolate. A total number of 987 mutants was collected from 25 isolates finally characterized. All the 25 isolates of *V. dahliae* produced both types of *nit* mutants, however, with different frequency. They were classified after phenotype test revealing 84–92% of *nit* 1/*nit* 3, and 8–16% of *nit* M mutants.

No mutants could be recovered from six isolates of *V. dahliae* and from four isolates of *V. longisporum*. The isolate-testers could be selected only from 15 isolates originating from The Netherlands (7), Poland (3), UK (3), Greece (1) and Spain (1).

Two isolates were self-incompatible. The *nit* mutants of these isolates were not able to make heterokaryon with the rest of their own complementary mutants, and with other isolates, and any VCGs testers.

**Table 1**

VCG testers of *Verticillium dahliae* from different countries

| Country | VCG designation | Isolate No. | Isolated from | Year of isolation | Tester strains | | |
|---|---|---|---|---|---|---|---|
| | | | | | *nit* 1 | *nit* M | *nit* M |
| Greece | 1 | 1V | *Gossypium* sp. | 1993 | $1V_{10}$ | $1V_3$ | |
| Greece | 1i | 7V | *Lycopersicon esculentum* | 1993 | $7V_4$ | $7V_6$ | |
| Greece | 1 | 30V | *Lycopersicon esculentum* | 1993 | – | $30V_3$ | |
| UK | β | 320[b] | *Fragaria ananassa* | 1985 | – | – | |
| UK | α | F4[1] | Soil | 1989 | – | D1 | |
| UK | α | F6[5] | Soil | 1989 | 5a | – | |
| USA | 1 | V44 | *Gossypium* sp. | – | 491 | 492 | |
| USA | 2A | PH | *Pistacia vera* | – | 495 | 496 | |
| USA | 2B | 115 | *Gossypium* sp. | – | 497 | 498 | |
| USA | 3 | PCW | *Capsicum* sp. | – | 499 | 500 | |
| USA | 3 | 70-21 | – | – | 501 | 502 | |
| USA | 4A | BB | *Solanum tuberosum* | – | 503 | 504 | |
| USA | 4B | S39 | Soil | – | 505 | 506 | |
| NL[b] | A.plat.II | 11 | *Acer platanoides* | 1993 | 2 | 20 | 21 |
| NL | es 95 | 18 | *Fraxinus excelsior* | 1988 | – | 20 | 34 |

After pairing which was repeated at least once, two isolates were assigned to VCG 2A, 13 isolates were assigned to VCG 2B, eight isolates to VCG 4B, and two isolates to VCG 4A (Table 2).

Cross-reactions occurred between isolates of VCG 2A and 2B. All the isolates included to VCG 2B, that formed strong heterokaryons with the VCG 2B tester, were able to make weaker but in some cases still strong heterokaryons with VCG 2A tester. Also VCG 2A isolates cross-reacted with VCG 2B tester, and VCG 4B isolates with VCG 4A tester. Similarly, two members of VCG 4B were also compatible to lesser strength with testers of VCG 3. Additionally, two representatives of VCG 2 (JKG 3a, LB PV-9) made equally strong heterokaryons with VCG 2B and VCG 1 testers, and weaker heterokaryons with VCG 2A tester. Despite of these interactions these isolates made very strong heterokaryons with corresponding English and Dutch testers. Therefore the isolate JKG 3a was included to VCG 2 (subgroup 2B).

Similarly, there were three bridge isolates which made heterokaryons differing in strength with representatives of both main groups VCG 2 and VCG 4.

**Table 2**

List of fungal isolates of *Verticillium dahliae* studied

| Isolate | Host plant | Origin | Source/year | VCG |
|---|---|---|---|---|
| 201 | *Acer platanoides* | The Netherlands | JAH/2000 | 4B |
| 3a | *Acer palmatum* | The Netherlands | JKG/2000 | 2B |
| 209 | *Chrysanthemum* sp. | The Netherlands | JAH/2000 | 2B |
| 214 | *Chrysanthemum* sp. | The Netherlands | JAH/2000 | 2B |
| 5a | *Solanum tuberosum* | The Netherlands | JKG/2000 | 4B |
| 12149a | *Solanum tuberosum* | UK | DCH/2000 | 4B |
| 237 | *Rosa* sp. | The Netherlands | JAH/2000 | 4B |
| 12a | *Rosa* sp. | The Netherlands | JKG/2000 | 4B |
| 1044a | *Fragaria vesca* | Poland | MRG/1999 | 2B |
| 12154a | *Fragaria vesca* | UK | DCH/2000 | 2B |
| 12077a | Soil | UK | DCH/1998 | 2B |
| 12081a | Soil | UK | DCH/1998 | 2B |
| 431 v-1 | *Olea europaea* | Greece | EP/2000 | 4A |
| C1F20R2a | *Olea europaea* | Spain | RJD/2000 | 4B |
| Manzanillo R-1a | *Olea europaea* | Spain | RJD/2000 | ND |
| 407 v-1 | *Gossypium* sp. | Turkey | EP/* | ND |
| 405 v-1 | *Gossypium* sp. | Turkey | EP/* | 2A |
| V 1791 | *Gossypium* sp. | Spain | RJD/1985 | 2A |
| 304 v-1 | *Gossypium* sp. | Greece | EP/2000 | ND |
| PV-9 | Soil | Spain | LB/* | 2B |
| PV-26 | Soil | Spain | LB/* | 2B |
| 287 v-1 | *Lycopersicum esculentum* | Spain | EP/2000 | ND |
| 1111a | *Prunus cerasus* | Poland | MRG/1999 | 2B |
| 12090a | *Humulus lupulus* | UK | DCH/1998 | 4B |
| 235 | *Catalpa* sp. | The Netherlands | JAH /2000 | 4B |
| 11a | *Syringa vulgaris* | The Netherlands | JKG/2000 | 4B |
| 1094a | *Berberis vulgaris* | Poland | MRG/2000 | 2B |
| V-006a | *Cucumis melo* | Spain | RJD/1999 | 2A |
| V-017a | *Cynara cardunculus* | Spain | RJD/1999 | ND |
| 402 v-1 | *Capsicum annuum* | Romania | EP/* | 2B |
| 351 v-1 | *Xanthium strumarium* | Greece | EP/2000 | ND |

*Unknown.

JAH – J.A. Hiemstra, The Netherlands, JKG – J.K. Goud, The Netherlands, DCH – D.C. Harris, UK, MRG – M. Rataj-Guranowska, Poland, EP – E. Paplomatas, Greece, RJD – R.J. Díaz, Spain, LB – L. Blanco, Spain.

ND – non determined.

## Discussion

The present study included 31 isolates of *V. dahliae* and four isolates of *V. longisporum*. The isolates were provided from seven countries, several laboratories and many different host-plants. The results presented here show that although the geographical and plant origin of isolates varied, the VCG diversity was rather limited.

The first group of isolates consisted of six representatives of *V. dahliae* and four of *V. longisporum*. They were not characterized as *nit* mutants could not be recovered from them. Zeise and von Tiedemann (2001) were unable to characterize *V. longisporum* for the same reason. It was not possible to generate mutants from several *V. dahliae* isolates from Greece and Spain, most probably because they were in bad condition. Altogether we had very few isolates in good shape from South Europe, probably their number was to small to find any representatives of VCG 1. This group is known to exist in the Mediterranean region of Europe. VCG 1 occurs sporadically in USA (Joaquim and Rowe 1990, Daayf et al. 1995) and in a few (not all) South European countries, like Spain (Collado-Romero et al. 2006) and Greece (Elena 1999). VCG 1 was noted among isolates from potatoes (USA), olive (defoliating type isolates) (Spain), cotton (USA, Spain, Greece) and artichoke (Spain). It has to be assumed that VCG 1 was not identified in Northern countries, like The Netherlands (Hiemstra and Rataj-Guranowska 2003), Poland (Rataj-Guranowska et al. 2002) or Germany (Zeise and von Tiedemann 2001) in Europe, and Canada (Dobinson et al. 1998) or Japan (Ebihara et al. 1999).

The present study is a continuation and confirmation of a work published in 2003 (Hiemstra and Rataj-Guranowska) concerning a group of *V. dahliae* isolates from The Netherlands. The present experiments were performed on a more differentiated group of isolates. However, the results obtained showed again that limited VCG differentiation in *V. dahliae* is not related to geographical location or plant origin of isolate.

There is one special aspect of the presented results that needs more careful study and discussion in the future. It concerns the cross-reactions occurrence. The presence of the bridge-isolates might be an evidence that testers for VCGs have not been selected properly. On the other hand, it could be an indication that VCGs are not completely genetically isolated. Such a phenomenon may change the present number of VCGs in *V. dahliae*.

## Streszczenie

ZGODNOŚĆ WEGETATYWNA W OBRĘBIE *VERTICILLIUM DAHLIAE* Z KILKU KRAJÓW EUROPEJSKICH

W badaniach zgodności wegetatywnej w obrębie *Verticillium dahliae* z Europy Północnej (Holandia, Polska, Wielka Brytania) i Południowej (Grecja, Hiszpania, Turcja, Rumunia) wzięto do doświadczeń grupę 31 izolatów. Składały się na nią

izolaty z roślin ważnych gospodarczo dla danego kraju (klon, chryzantema, bawełna, oliwka, ziemniak, róża, truskawka, pomidor) oraz z mniej ważnych (karczoch, berberys, katalpa, wiśnia, chmiel, bez, melon, papryka, *Xanthium*) i z gleby. Pary zgodnych mutantów *nit* zostały starannie wyselekcjonowane z każdego izolatu i zastosowane w krzyżowaniu wszystkich izolatów ze sobą. Ponadto izolaty te były skrzyżowane z międzynarodowymi testerami VCG z USA, Holandii, Grecji i Wielkiej Brytanii.

Wyniki wskazują na ograniczoną różnorodność VCG w Europie. Zidentyfikowano tylko dwie grupy VCG: VCG 2 (podgrupy 2A i 2B) i VCG 4 (podgrupy 4A i 4B). Jednak wszystkie izolaty z podgrup 2A lub 4A były równocześnie zgodne, aczkolwiek słabiej, z testerami grup odpowiednio 2B lub 4B. To samo dotyczyło izolatów z podgrup 2B i 4B. Wystąpiły trzy tzw. mostowe izolaty, które tworzyły zróżnicowane heterokariony z testerami obu głównych grup. Dwa izolaty były słabo zgodne z testerem VCG 3, za to silnie z testerem VCG 4. Podobnie jeden izolat był słabo zgodny z VCG 1, za to silniej z VCG 2. Sześciu izolatów, głównie z Hiszpanii i Grecji, nie udało się scharakteryzować ze względu na brak mutantów *nit* lub ich rewersje do stanu dzikiego. Nie odzyskano także mutantów z czterech izolatów *V. longisporum*. Łącznie spośród 25 scharakteryzowanych izolatów 15 było w VCG 2, a 10 w VCG 4. Izolaty z drzew i krzewów były w obu grupach, co jest wskazówką, że właśnie reprezentanci tych grup powinni być uwzględniani w testach infekcyjnych w krajach europejskich.

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