

Differences in phenotypic stability and adaptive variation between the main European and American lineages of *Phytophthora ramorum*

Clive Brasier, Susan Kirk and Joan Rose

Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK
Correspondence: clive.brasier@forestry.gsi.gov.uk

Introduction

Outbreaks of stem and foliar disease on trees and ornamental nursery stock, caused by *Phytophthora ramorum*, are occurring in California and Oregon, USA and in much of Europe. In the USA the pathogen is epidemic on a variety of oaks, oak relatives and shrub species (Rizzo and Garbelotto, 2003) and a range of nursery species (Davidson *et al.*, 2003). In Europe it has been widely distributed through the nursery trade on rhododendrons and viburnums and has recently spread into the wild in south-west Britain and the Netherlands (Brasier *et al.*, 2004; and cf. Brasier and Jung, Chapter 1; Goheen and Hansen, Chapter 2). Both the American and European outbreaks are believed to have resulted from the introduction of the pathogen, via the plant trade, over the past 10–20 years. However the geographic centre of origin of *P. ramorum* remains unknown. Suggestions as to its source include Yunnan (western China), the Himalayas and Taiwan (Brasier *et al.*, 2004).

European (EU) and North American (NA) isolates of *P. ramorum* have identical isozyme profiles (W. Man in't Veld, personal communication, summarized in Poster 33) and identical ITS profiles, indicating their conspecificity. Studies on AFLP and microsatellite polymorphisms show that *P. ramorum* has three distinguishable molecular clades. These exhibit only limited molecular variation and consist of a small number of closely related AFLP and microsatellite genotypes (Garbelotto *et al.*, Chapter 39; Prospero *et al.*, 2004; Ivors *et al.*, 2004, 2006; Kroon *et al.*, 2004). All European isolates to date comprise one polymorphic clade. The majority of North American isolates, including all those from forest ecosystems, comprise another polymorphic clade. Rare isolates from a nursery in Washington State, Pacific Northwest comprise a third, monomorphic clade (Ivors *et al.*, 2006). Recently, isolates of the main European clade were also discovered in the same nursery in Washington (Hansen *et al.*, 2003; Ivors *et al.*, 2006).

In view of these observations it was recently (2006) informally agreed that, for the present:

- the predominant European clade of *P. ramorum* be designated the European 1 lineage, or EU1;
- the predominant North American clade be designated the North American 1 lineage, or NA1 (cf. Rizzo *et al.*, 2005);
- the rare third clade from the Washington nursery be designated the North American 2 lineage, or NA2.

This chapter has been updated to take account of these changes in *P. ramorum* terminology. The limited molecular variation shown by each of the three lineages is consistent with their being products of separate introduction events from an original, more variable gene pool (Ivors *et al.*, 2006).

Studies on the sexual breeding system have also revealed differences. *P. ramorum* is apparently A1/A2 heterothallic (Werres *et al.*, 2001) and therefore potentially outcrossing. To date, virtually all EU1 lineage isolates tested have been of A1 sexual compatibility type (mating type); whereas all NA2 lineage isolates have been exclusively of A2 type (Werres *et al.*, 2001; Werres and Zielke, 2003; Brasier and Kirk, 2004). A single EU1 lineage isolate of A2 compatibility type has been found – in Belgium (Werres and De Merlier, 2003). However in pairings of *P. ramorum* with A1s and A2s of other *Phytophthora* species, and in pairings between EU1 A1 and NA1 A2 *P. ramorum* isolates, production of gametangia is exceptionally slow, sparse and unpredictable (Brasier and Kirk, 2004). It therefore remains unclear whether *P. ramorum* is an actively sexually outcrossing species.

Continuous variables such as pathogenic aggressiveness are good indicators of adaptive differences between isolates, lineages or populations (Brasier, 1999). In contrast to molecular studies, preliminary studies on behavioural characters such as aggressiveness revealed unexpectedly large differences between the EU1 and NA1 lineages of *P. ramorum* (Brasier, 2003). Therefore, in support of plant health policy, the comparative risk to trees posed by the EU1 and NA1 lineages of *P. ramorum* in terms of variation in continuous characters was investigated. One objective was to investigate whether behavioural differences between them warranted preventing the introduction of NA1 lineage isolates into Europe or vice versa. In particular whether new genotypes, e.g. more aggressive genotypes or genotypes with different host

specificities, might arise if genetic recombination were to occur between the two lineages. Another objective was to assess whether differences between the EU1 and NA1 lineages were sufficient to indicate they were discrete sub-populations, rather than components of a single cohesive species. The studies were undertaken bearing in mind that natural selection acts on the phenotype, not on the genotype, and that molecular markers like those used to distinguish EU1 and NA1 lineages are usually adaptively neutral (Brasier, 2003).

Methods and results

Samples of European (EU1) and North American (NA1) lineage isolates were compared for continuous variation across different environments or stress conditions (gene \times environment or G \times E tests: cf. Brasier, 1999). Variables examined included growth rate, phenotypic stability, host range and pathogenic aggressiveness. The isolates were chosen from within a pool of 110 European isolates obtained from shrubs and trees in six different European countries in 2003–04 (see Brasier and Kirk, 2004); and from 68 North American isolates obtained from trees and shrubs in California and Oregon kindly provided by D. M. Rizzo and E. M. Hansen between 2002 and 2004.

Slower growth rate of NA1 lineage isolates

Growth rate on carrot agar (CA) was the first continuous variable examined. During 2002–03 a series of linear growth rate tests was conducted on CA at 20°C, near the growth optimum for *P. ramorum* (Werres *et al.*, 2001); 30–39 isolates were used per population sample. The EU1 isolates grew significantly faster on average than the NA1 isolates in all tests. In some tests a total

separation of the EU1 and NA1 samples occurred. For example, in the second test (Figure 1) the radial growth rate of 30 EU isolates averaged 3.62 ± 0.08 mm day⁻¹ and 39 NA isolates only 2.70 ± 0.33 mm day⁻¹. The growth rate of the EU isolates ranged from 3.45 to 3.75 mm day⁻¹ while that of the NA isolates was much more variable at 2.09–3.56 mm day⁻¹. In the third test (Figure 2) an EU1 sample again grew faster and did not overlap with the NA1 sample, even though the NA1 sample comprised 30 isolates freshly obtained from the field and the EU1 isolates had been in culture > 2 years. The NA1 isolates were again much more variable than the EU1 isolates in this test. These results indicated adaptive differences between the EU1 and NA1 lineages.

Similar tests with 35 EU1 and 39 NA1 isolates were conducted on CA at 12.5, 15, 25 and 27°C, i.e. at supra- and sub-optimal temperatures for *P. ramorum*. Such temperatures could put the pathogen under greater environmental stress and would therefore be expected to reveal any wide genetic divergence among individual isolates or populations. The EU1/NA1 growth rate difference was maintained throughout (Figure 3). The EU1 isolates always grew significantly faster, on average, than the NA1 isolates; and again, in several tests, a complete separation of the EU1 and the NA1 samples occurred. At the same time, the shapes of the mean temperature growth curves for the EU and NA samples across all four temperatures tested (12.5, 15, 20 and 27°C) were very similar. This indicates that although the EU1 and NA1 lineages differ markedly in growth rates, they nonetheless show a very similar response to temperature. This is consistent with their being conspecific.

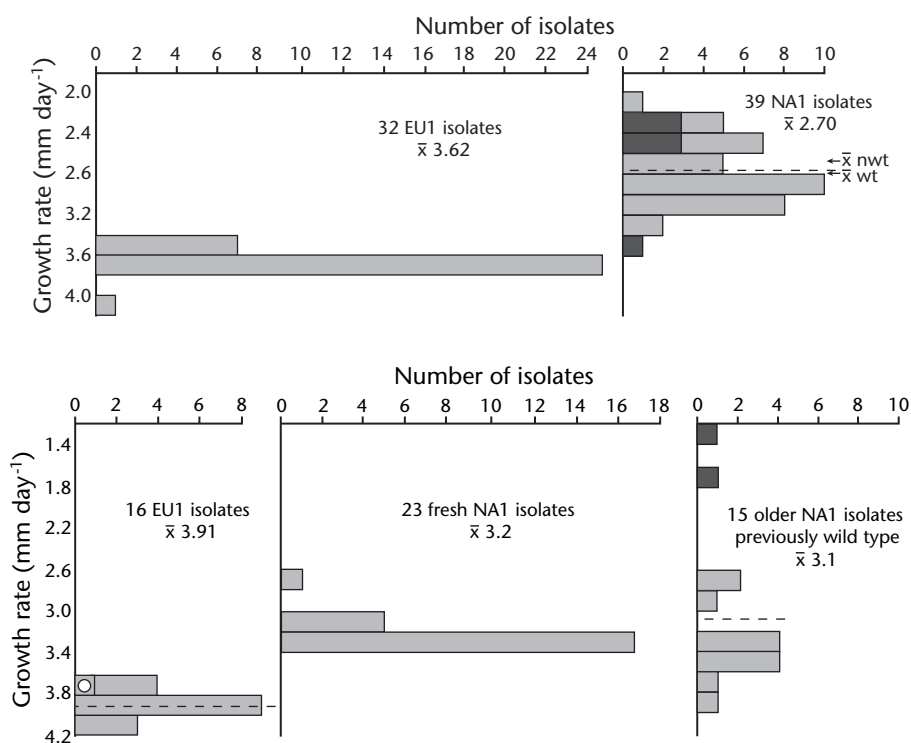


Figure 1 Comparative growth rates of 30 EU1 and 39 NA1 lineage isolates at 20°C. Dashes indicate overall means. Dark hatching: non-wild-type isolates.

Figure 2 Comparative growth rates of 23 fresh NA1 isolates, 15 older NA1 isolates and 16 older EU1 isolates at 20°C. Dashes indicate overall means. Dark hatching: non-wild-type isolates. White spot: Belgian EU1 A2 isolate.

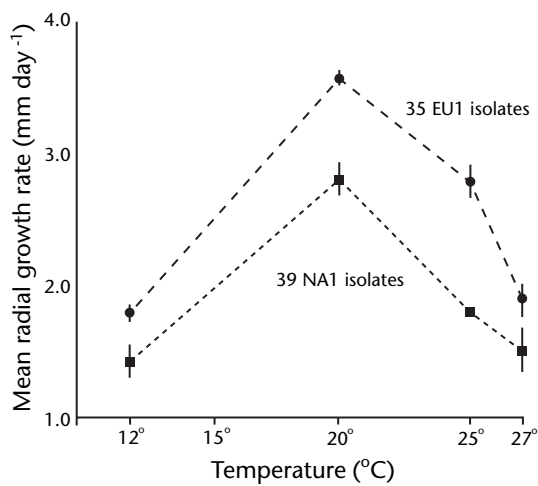


Figure 3 Mean growth curves of 35 EU1 and 39 NA1 lineage isolates across five different temperatures. Bars indicate SEs.

Comparative growth rates in other environments

Growth tests were also carried out in other stress environments. These included exposure to continuous light; and a range of optimal (1.5%) and supra-optimal (3, 4 and 5%) agar concentrations to simulate decreased free water levels, using CA at 20°C as the basic conditions. In the agar concentration tests growth rates of all isolates decreased markedly between the 1.5 and 3% agar levels but did not decrease further above 4%. EU1 isolates grew significantly faster, on average, than the NA1 isolates under all light and agar conditions tested. Otherwise there was no evidence of a differential response. These results were again consistent with EU1 and NA1 lineages being conspecific, but having adaptive differences. Growth tests were also conducted on samples of EU1 and NA1 isolates at between 28 and 31°C, close to the

P. ramorum's upper temperature limit for growth and therefore representing high stress conditions. None of the isolates grew at 31°C. At 30°C, only 37% (12/35) of EU1 isolates grew, whereas 80% (31/39) of NA1 isolates did so. This indicates that EU1 lineage isolates have a slightly lower maximum temperature limit for growth than NA1 isolates, suggesting a minor adaptive difference.

Phenotypic instability of NA1 lineage isolates

In growth rate tests the variance (σ^2) of the EU1 samples tended to be very small (cf. Figure 1), indicating near isogenicity or clonality for 'adaptive' genes governing growth rate. That of the NA1 samples was much larger, suggesting greater variability in these genes. When colony morphologies of the EU1 and NA1 isolates were examined following the growth rate tests, EU1 isolates were found to be of a rather uniform and characteristic 'wild type' (*wt*) colony morphology (Figure 4), consistent with their limited growth rate variation. NA1 isolates were either of similar *wt* morphology, or fell into a range of morphologically variable, often slow growing 'non-wild type' (*nwt*) colony types (Figure 5).

In the growth rate distributions shown in Figures 1 and 2 *nwt* isolates are distinguished from *wt* isolates by dark hatching. It should be noted that in the growth rate test shown in Figure 2, the sample of 15 older NA1 isolates was selected on the basis that they had all been of *wt* morphology in the previous growth rate test (Figure 1), i.e. *nwt* isolates were deliberately excluded. However, two of these previously *wt* isolates now grew as *nwt* (Figure 2) and also retained their *nwt* phenotype subsequently. This demonstrated that NA1 *wt* isolates could degenerate into *nwt* type, further emphasizing their instability.

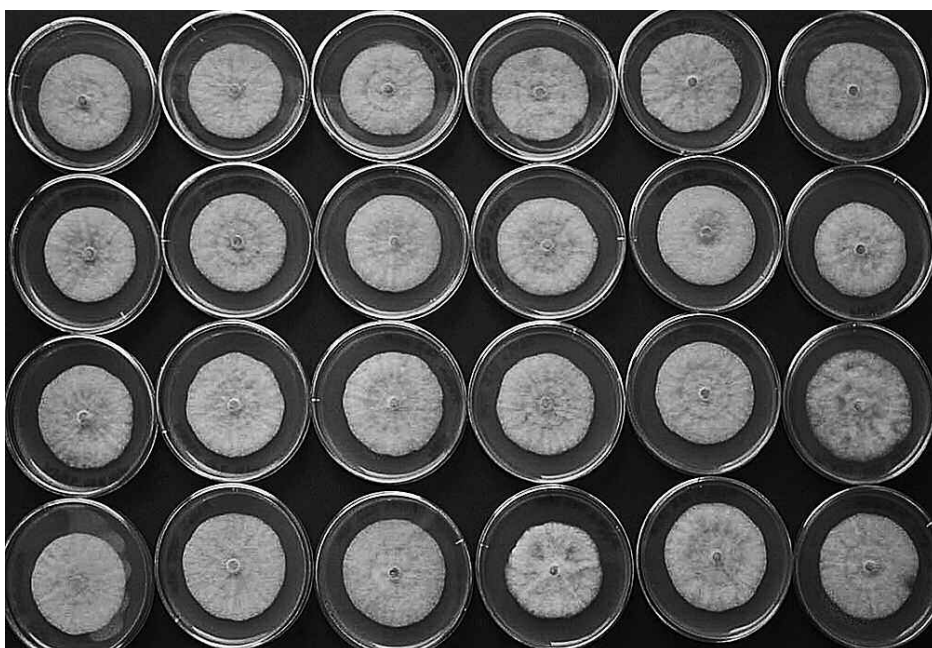
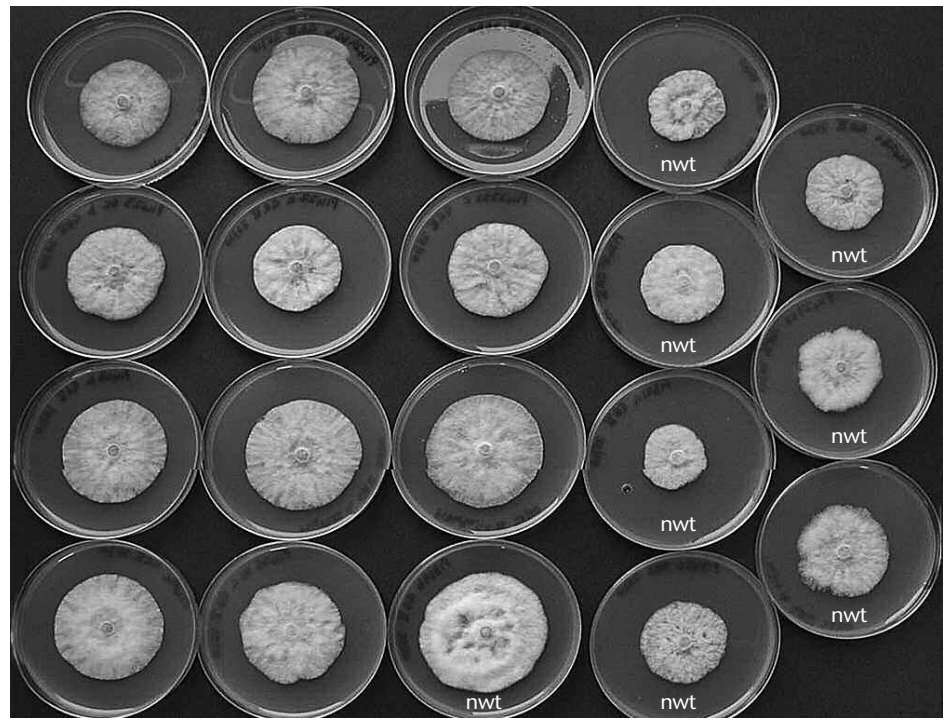


Figure 4 Colony types on CA of 24 EU1 lineage isolates from six countries, showing relative uniformity.

Figure 5 Colony types of 19 NA1 lineage isolates on CA. Note variation in colony patterns and growth rates including one fast-growing *nwt* colony (see also Figure 1); *nwt*: non-wild-type colonies.

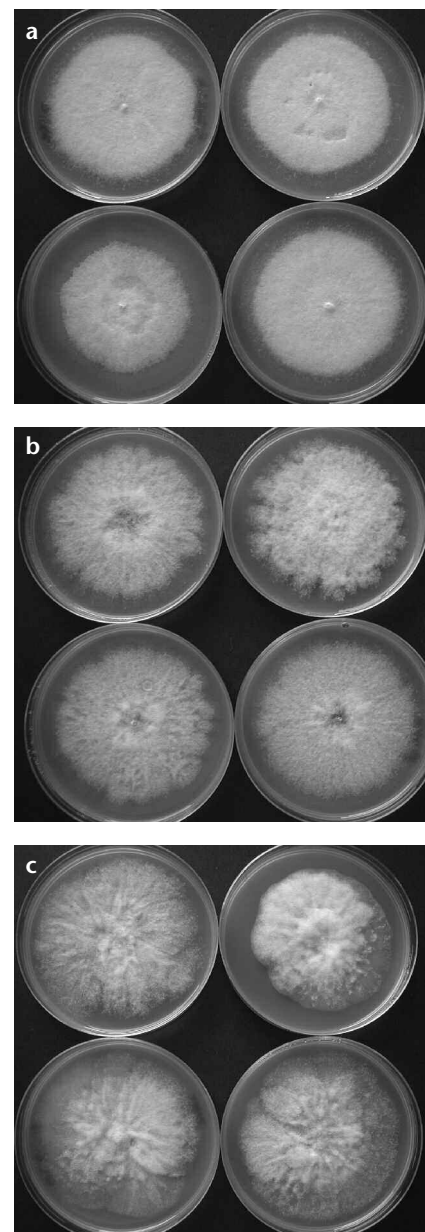


To examine this process further, single hyphal tip subcultures were taken from selected EU1 and NA1 *wt* isolates and from NA1 *nwt* isolates and their colony patterns examined. The subcultures from EU1 *wt* isolates remained *wt*, i.e. they were intrinsically rather stable (Figure 6a). In contrast, the subcultures from the NA1 isolates were often intrinsically unstable. Subcultures from NA1 *nwt* colonies produced mostly rather variable and unstable *nwt* forms (Figure 6c). Even those from NA1 *wt* colonies yielded degenerate, unstable *nwt* forms as well as some *wt* forms (Figure 6b). The genetic basis of this unusual developmental instability in NA1 isolates is unknown. Virus infection was considered as one possible explanation. Six *nwt* isolates were therefore screened for viral dsRNA (C. Hacker, C.M. Brasier and K.W. Buck, unpublished) but no dsRNA was detected.

Cultural phenotype of the Belgian A2 isolate

The single *P. ramorum* isolate from Belgium shown by S. Werres (personal communication) to be of A2, not A1 sexual compatibility type (see Introduction), was compared with 30 EU1 and 39 NA1 isolates for its growth rate and its colony type on CA at 20°C. It was shown to be a typical EU1 isolate in terms of its growth rate (Figure 2) and it had a typical EU1 *wt* colony type, i.e. it appeared to be no different phenotypically from other EU1 isolates.

Figure 6 Representative colonies of *P. ramorum* isolates on CA derived as single hyphal tip subcultures. (a) Subcultures from EU1 wild-type isolate P1242. (b) Subcultures from NA1 wild-type isolate P1404. (c) Subcultures from NA1 non-wild-type isolate P1427. Note the increasing level of instability from (a) through (c).



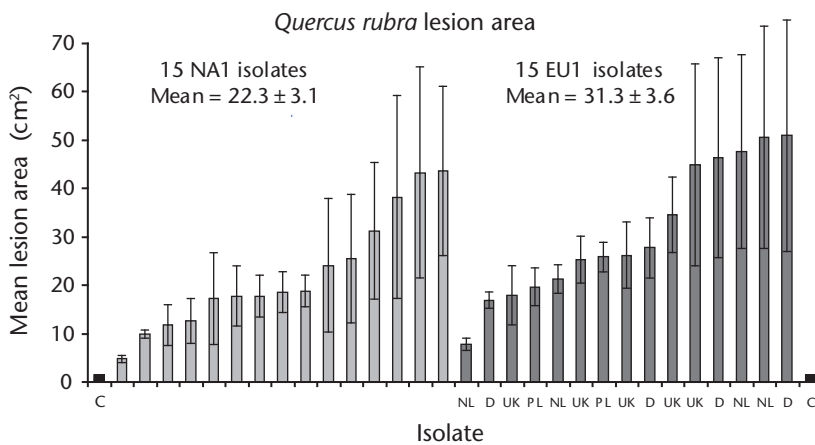


Figure 7 Comparative aggressiveness of 15 EU1 and 15 NA1 wild-type isolates of *P. ramorum* on *Q. rubra*. Bars indicate SEs. NL: Netherlands, D: Germany, UK: United Kingdom, PL: Poland; C: agar control.

Lower pathogenic aggressiveness of NA1 lineage isolates

In 2002 and 2003 three experiments were conducted to compare the aggressiveness of EU1 and NA1 isolates in the phloem (inner bark) of the susceptible tree host *Quercus rubra*. Fresh cut lower stems c. 20 cm diameter were wounded inoculated with 8–15 EU1 and 8–15 NA1 isolates using the method of Brasier and Kirk (2001). The logs were then sealed in polythene bags and incubated in a quarantine chamber at 20°C. Resulting lesion areas were measured after 5–6 weeks (method of Brasier and Kirk, 2001).

The first aggressiveness experiment involved 8 EU1 and 8 NA1 isolates chosen at random from an early growth rate test. Among the NA1 isolates 7 exhibited non-wild-type (*nwt*) colonies and 1 wild type (*wt*). The overall mean lesion area of the EU1 isolates, at 36.8 ± 2.7 cm², was significantly greater than that of the NA1 isolates, at 14.00 ± 1.8 cm² ($P < 0.001$).

In the first aggressiveness experiment most NA1 isolates were *nwt*, which tend to be slower growing than *wt* isolates. The experiment was therefore repeated using 15 EU1 isolates (from four countries) and 15 NA1 isolates (across hosts and locations in California), but with 14 of the latter pre-selected to be *wt* on the basis of the growth test in Figure 1. A similar result was obtained, though the ranges of the two groups

overlapped closely (Figure 7). Mean lesion areas were: EU1 isolates, 31.3 ± 3.6 cm² and NA1 isolates 22.3 ± 3.1 cm² ($P = 0.098$; *t*-test). There were also significant differences in aggressiveness between isolates with both groups (Figure 7).

The 15 NA1 isolates tested in the above experiment, though of *wt* colony type, were all relatively old isolates that had been in culture since early in 2001. In September 2003, 15 EU1 isolates (the same as in Figure 2) and 15 fresh NA1 isolates (all NA1 wild types from Oregon and California; see also Figure 2) were inoculated into *Q. rubra*. The main objective was to confirm whether the fresh NA1 isolates behaved in the same way as the older NA1 isolates. Although the ranges of the two groups overlapped closely (Figure 8), the mean lesion area of the EU1 isolates, at 79.8 ± 3.1 cm², was again significantly higher than that of the NA1 isolates, at 60.5 ± 2.6 cm² ($P < 0.001$). There was also significant variation within both the EU1 and NA1 groups.

These experiments demonstrated that European 1 lineage isolates were on average more aggressive than North American 1 lineage isolates, whether or not the NA1 isolates were freshly collected *wt* isolates or more variable older *nwt* isolates. This provides evidence of additional adaptive differences between the two lineages. Growth rate and aggressiveness were not

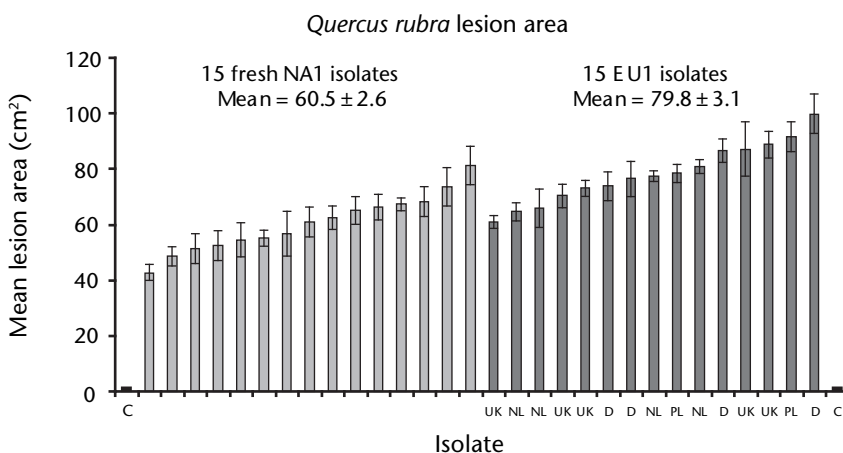


Figure 8 Comparative aggressiveness of 15 fresh NA1 isolates and 15 older EU1 isolates of *P. ramorum* on *Q. rubra*. Bars indicate SEs. NL: Netherlands, D: Germany, UK: United Kingdom, PL: Poland; C: agar control.

found to be correlated (data not shown). Hence at least partially different sets of genes may be involved in each of these characters.

Host range comparison

The potential host ranges of EU1 and NA1 isolates were compared by wound inoculation of mature stems of 23 different European and North American tree species. Comparisons were based on average lesion sizes after 5 weeks using 2 EU1 and 2 NA1 isolates with c. 8–10 replicate inoculation points per isolate. Five of the tree species were assessed as resistant, 10 as 'less susceptible' and eight as 'more susceptible'. The pattern of susceptibility of the 23 tree species to the EU1 and NA1 isolates was very similar, yet distinct from the susceptibility pattern to other *Phytophthora* species. This is again consistent with the EU1 and NA1 lineages being conspecific.

Discussion

The two main molecular lineages of *P. ramorum* found in North America and Europe, NA1 and EU1, probably represent different founder genotypes from a more variable ancestral *P. ramorum* gene pool in the pathogen's geographic centre of origin. It is likely that each lineage became established as a result of a genetic bottleneck during an introduction event (cf. Brasier, 2003; Ivors *et al.*, 2004); each subsequently spreading as a molecular near-clone.

Adaptive variation in the lineages

The G x E tests on the EU1 and NA1 lineages described here involved measurement of continuous characters across a range of stress environments. They show that the two lineages share important behavioural responses, such as the shape of their growth-temperature curves, their upper temperature limit for growth and their host range profiles. Such similarities are consistent with conspecificity. Indeed, they are unsurprising in view of other major characters shared by European and North American isolates, such as unusually large chlamydospores, large caducous sporangia, a somewhat idiosyncratic sexual breeding system and identical ITS rDNA sequences and isozyme profiles. Collectively, these define *P. ramorum* as a unique evolutionary entity: the EU1 and NA2 lineages appear to be adapted to a broadly similar life style and to belong to the same major phylogenetic unit.

Equally however, the tests have revealed important differences in the behaviour of EU1 and NA1 isolates, including their mean growth rates, their intrinsic levels of colony stability and their pathogenic aggressiveness. Therefore, although the EU1 and NA1 lineages are near-clonal at the neutral DNA level, each lineage is also phenotypically distinct. Notably, NA1 is on average both slower growing and less aggressive than EU1.

Furthermore, within each lineage there is significant variation in aggressiveness, and NA1 exhibits a considerable variation for growth rate. The extent of these differences was unexpected. They are likely to be controlled by underlying differences in multiple genes and to reflect differences in fitness, i.e. to be adaptive differences. They are supported by other differences between the EU1 and NA1 lineages, such as their being of predominantly A1 and A2 sexual compatibility types respectively, and their different AFLP and microsatellite profiles.

That molecular clones of *Phytophthora* can exhibit marked phenotypic variation has been shown previously for *P. cinnamomi* and *P. infestans*, where it has been ascribed to mitotic or somatic recombination (Huberli *et al.*, 2001; Dobrowolski *et al.*, 2003; Goodwin *et al.*, 1994). It suggests that patterns of adaptive genes may be evolving at a different rate from patterns of neutral molecular DNA in *Phytophthora* (Brasier, 2003). In the present case, it may reflect a combination of genetic differences existing between the EU1 and NA1 lineages at the time of their introduction into Europe or North America; adaptive variation arising through somatic recombination at heterozygous loci; and different selection pressures the two lineages have experienced since their introduction. For example, growth rate variation in the EU1 population may have remained small because this population has been subject to intense directional or stabilizing selection within the more uniform European nursery environment. That of the NA1 population could be larger because the latter has been exposed to a more heterogeneous set of environments – or selection pressures – in American forests (Brasier, 2003).

A striking feature of the NA1 lineage is its intrinsic phenotypic instability, as revealed by colony development. In contrast to EU1 isolates, which were rather stable *wt* types, NA1 isolates were both of wild-type and of phenotypically variable *nwt* types. Even NA1 isolates that were *wt* when first isolated exhibited a marked tendency to become unstable and *nwt*. It seems reasonable, as a working hypothesis, that this intrinsic instability of NA1 isolates is in some way also a facet of their lower growth rate and aggressiveness. It is also reasonable to suppose that this instability may occur under field as well as laboratory conditions. In nature such degenerate forms would probably be at a fitness disadvantage and be strongly selected against. As such they would be less likely to be recovered from host material than NA1 wild types.

The genetic basis of this instability is unknown. In a preliminary screening, six different NA1 lineage non-wild-type isolates were examined for virus infection. No viral dsRNA was detected (C. Hacker, K.W. Buck and C.M. Brasier, unpublished observations). The instability could involve the effects of other deleterious

cytoplasmic genes such as senescence plasmids. Another explanation could be mitotic recombination, leading to an unbalanced nuclear genome. The chances of such genetic imbalance could be heightened if the NA1 lineage had previously undergone a level of introgression. Indeed, similar colony instability has been observed in some genotypes of the interspecific hybrid *P. alni* (Delcan and Brasier, 2001; Brasier *et al.*, 2004).

The single known A2 sexual compatibility isolate from Belgium grouped with the EU1 lineage with regard to its growth rate and colony type. This isolate has also been shown to conform to the EU1 lineage in terms of its molecular profile (Ivors *et al.*, 2004; Kroon *et al.*, 2004). Therefore, it may well be near isogenic to other European 1 lineage isolates, apart from its A2 locus. In this case the present data give further support to the proposal of Ivors *et al.* (2004) that this A2 isolate has arisen locally from an EU1 A1 type via a rare somatic recombination event.

Variation through sexual or somatic recombination

Present evidence from *in vitro* pairing studies indicates the A1/A2 sexual compatibility system of *P. ramorum* is somewhat atypical (Brasier and Kirk, 2004). Sexual recombination in nature between A1s and A2s cannot therefore be assumed to be an automatic outcome should they come into contact. However, there is also the possibility of somatic recombination between A1s and A2s, e.g. via zoospore fusion. Recently, isolates of the European 1 lineage were found in North American nurseries (Hansen *et al.*, 2003; Ivors *et al.*, 2006). This indicates that continuing international exchange of plant material has brought the EU1 and NA1 lineages into contact, allowing the possibility of genetic exchange (Rizzo *et al.*, 2005). In view of the phenotypic differences between the NA1 and EU1 lineages described here, if recombination does occur between them there must be a risk that significant additional allelic variation would be generated, leading to novel adaptive variation. This argues for preventing the introduction of the NA1 lineage into Europe.

As discussed above, in Belgium both A1 and A2 types of the EU1 lineage are present. They may also be near isogenic. Nonetheless, since Phytophthoras are diploid organisms, sexual or somatic recombination between these EU1 A1s and A2s could well release some novel adaptive phenotypic variation via genetic recombination at heterozygous loci.

Acknowledgements

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References

- Brasier CM (1999). Fitness, continuous variation and selection in fungal populations: an ecological perspective. In: *Structure and dynamics of fungal populations*, ed. J Worrall. Kluwer Academic Publishers, Dordrecht, 307–339.
- Brasier CM (2003). Sudden oak death: *Phytophthora ramorum* exhibits transatlantic differences. *Mycological Research* **107**, 258–259.
- Brasier CM and Kirk, SA (2001). Comparative aggressiveness of standard and variant hybrid alder Phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. *Plant Pathology* **50**, 218–229.
- Brasier CM and Kirk SA (2004). Production of gametangia by *Phytophthora ramorum* *in vitro*. *Mycological Research* **108**, 823–827.
- Brasier, CM, Kirk, SA, Delcan, J, Cooke, DEL, Jung, T and Man in't Veld, WA (2004). *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrids spreading on *Alnus* trees. *Mycological Research* **108**, 1172–1184.
- Brasier CM, Denman S, Brown A and Webber J (2004). Sudden oak death (*Phytophthora ramorum*) discovered on trees in Europe. *Mycological Research* **108**, 1108–1110.
- Davidson, JM, Werres S, Garbelotto M, Hansen EM and Rizzo DM (2003). Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Online. *Plant Health Progress* doi:10.1094/PHP-2003-0707-01-DG.
- Delcan J and Brasier, CM (2001). Oospore viability and variation in zoospore and hyphal tip derivatives on the hybrid alder Phytophthoras. *Forest Pathology* **31**, 65–83.
- Dobrowolski MP, Tommerup IC, Chearer BL and O'Brien PA (2003). Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. *Phytopathology* **93**, 695–704.
- Goodwin SB, Cohen BA and Fry WE (1994). Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences, USA*, **191**, 11591–11595.
- Huberli D, Tommerup IC, Dobrowolski MP, Calver MC and Hardy GE St J (2001). Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research* **105**, 1053–1064.
- Ivors KL, Hayden K, Bonants PJM, Rizzo DM and Garbelotto M (2004). AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. *Mycological Research* **108**, 378–392.
- Ivors KL, Garbelotto M, Vries IDE, Ruyter-Spira C, Hekkert BTE, Rosenweig N and Bonants PJM (2006). Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. *Molecular Ecology* **15**, 1493–1505.
- Kroon LPNM, Verstappen ECP, Kox LFF and Bonants PJM (2004). A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum*. *Phytopathology* **94**, 613–620.
- Prospero S, Black JA and Winton LM (2004). Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death. *Molecular Ecology Notes* **4**, 672–674.
- Rizzo DM and Garbelotto M (2003). Sudden oak death: endangering California and Oregon forest ecosystems. *Frontiers in Ecology of the Environment* **1**, 197–204.

Rizzo DM, Garbelotto M and Hansen EM (2005). *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. *Annual Review of Phytopathology* **43**, 309–335.

Werres S, Marwitz R, Man In'T Veld WA, De Cock AWAM and Bonants PJM (2001). *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* **105**, 1155–64.

Werres S and Zielke B (2003). First studies on the pairing of *Phytophthora ramorum*. *Journal of Plant Disease and Protection* **110**, 129–130.

Werres S and De Merlier D (2003). First detection of *Phytophthora ramorum* mating type A2 in Europe. *Plant Disease* **87**, 1266.