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Aggressiveness of *Phytophthora infestans* on detached potato leaflets in four Nordic countries

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Potato fields in Denmark, Finland, Norway and Sweden were sampled for single-lesion isolates of *Phytophthora infestans*. The aggressiveness of the isolates was determined on detached leaflets of potato cvs Bintje (susceptible) and Matilda (moderately resistant). The aggressiveness tests were carried out in the respective home countries of the isolates, with the exception of the Danish isolates. Fifteen Danish isolates were studied in each of the other three countries, including five isolates tested in all three laboratories. Results obtained from the Danish isolates revealed substantial differences between the test laboratories for infection efficiency, lesion growth rate and sporulation capacity on detached leaflets. When the laboratory effect was taken into account, the differences in aggressiveness between the countries were generally small or inconsistent between the test cultivars and epidemiologically insignificant. By contrast, variation among isolates within countries was substantial. The magnitude of the variation depended on country and cultivar. Maximal variation for the means of the isolates was between 89 and 185 h for latent period, between 100 and 1297 sporangia mm^{-2} for sporulation capacity and between nearly zero and 6 mm day^{-1} for lesion growth rate. Typically less than 1% of sporangia were able to cause infections, except in Norway. These extraordinarily low values may be an artefact of the testing method. High variation in results between the test laboratories emphasizes the need for caution when comparing results obtained by different research groups.

Keywords: epidemiology, infection efficiency, lesion growth, potato late blight, sporulation capacity

Introduction

Phytophthora infestans, the causal agent of potato late blight, is a heterothallic oomycete with two mating types, A1 and A2. From the mid-19th century to the end of the 1970s, European *P. infestans* populations were dominated by a single clonal lineage possessing only the Ib mitochondrial haplotype and A1 mating type (May & Ristaino, 2004). During the 1980s, a diverse population of *P. infestans* originating in the New World, probably from Mexico, replaced the old clonal lineage in Europe (Fry *et al*., 1993). One possible reason for the displacement was higher fitness of the migrated genotypes in terms of more frequent metalaxyl resistance and higher aggressiveness to the potato crop (Shattock, 2002). The immigrant population also contained both mating types, which enabled sexual reproduction through formation of oospores. Sexual reproduction has been suggested as being responsible for the continuous appearance of new genotypes, especially in the Netherlands and Nordic countries (Drenth *et al*., 1994; Brurberg *et al*., 1999) resulting in high phenotypic variation (Hermansen *et al*., 2000; Lehtinen *et al*., 2007, 2008).

The population change has also affected the epidemiology of potato late blight in Europe. Epidemic onset has become earlier, partly as a result of oospores as a new inoculum source (e.g. Widmark *et al*., 2007), and the general aggressiveness of the *P. infestans* populations has increased (Day & Shattock, 1997; Flier & Turkensteen, 1999). These changes have led to increased use of fungicides to control the disease (Fry & Goodwin, 1997; Hannukkala *et al*., 2007). The need for repeated preventative fungicide applications throughout the growing season as a consequence of the polycyclic nature of the disease makes commercial potato production heavily dependent on fungicides. At the same

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time, public demand for a reduction in the use of pesticides in agriculture is growing stronger. One solution for this dilemma has been the utilization of decision support systems (DSS) designed to alert growers when weather favours epidemic development, and in this way enable the use of fungicides only for justified needs. However, most of the existing DSSs are based on epidemiological data obtained well before the population change (Harrison, 1992). The increase in geno- and phenotypic variation in Nordic countries raises the question of whether the aggressiveness of the *P. infestans* populations has also changed (Brurberg *et al.*, 1999; Lehtinen *et al.*, 2008).

The original definition of aggressiveness was stated by Vanderplank (1963) as the quantity of disease caused by a pathogenic strain on a susceptible host. By this definition, aggressiveness is a quantitative result of a pathogen–host interaction. The environment (temperature, light, relative humidity, etc.) will have a large influence on the outcome of this interaction, as will a number of traits of the pathogen, such as spore production and growth rate in the host. The usual way of assessing aggressiveness is to measure variation in pathogen-dependent components while keeping host and environmental influences constant. This has been done extensively for *P. infestans* (Day & Shattock, 1997; Flier & Turkensteen, 1999; Lebreton *et al.*, 1999; Carlisle *et al.*, 2002).

The objective of this study was to determine aggressiveness components of the current Nordic *P. infestans* population to be used in submodels for DSSs. The variation in these components between and within the four Nordic countries was studied to ascertain if different values of aggressiveness components are needed in DSSs for each country. The practical experimental work was shared between three laboratories. One key aim was to examine if measurements of aggressiveness components between different laboratories can be made consistent by harmonizing methods and by managing experimental errors using a sophisticated experimental design.

Materials and methods

Isolates

In 2003, isolates of *P. infestans* were collected in Denmark, Finland, Norway and Sweden. The sampling and isolation of the isolates were described in Lehtinen *et al.* (2008). The sampling was targeted to major potato-growing areas within each country. Isolates were collected when approximately 10% of the leaf area in the field was affected by blight. This was done to let natural selection increase the seasonal fitness of the collected isolates, but still allow for sampling of single lesions. Leaflets were picked individually and placed in small plastic bags. Samples collected by local advisors were mailed in envelopes to the participating research institutes. Altogether, 61 isolates were obtained from Denmark, 264 from Finland, 329 from Norway and 89 from Sweden.

Twenty-five single-lesion isolates per country were selected for aggressiveness tests that were carried out in Norway, Sweden and Finland. Fifteen Danish isolates were sent to each of the three countries and of these 15, five were tested in all three countries. The isolates were selected to represent the sampled geographical area as well as possible. Therefore, equal numbers of isolates were randomly picked from each sampled potato-production area. The selected isolates originated from separate fields. Mating type, virulence pathotype and response to the fungicides metalaxyl and propamocarb were determined in a previous study (Lehtinen *et al.*, 2008). In Norway, the tests were carried out during the first half of 2004, in Sweden during the second half of 2004 and in Finland the first replicate was started in May and the last finished in October 2004.

Isolation and inoculum production

Spores from single lesions were transferred to tuber slices and, after incubation, hyphal tips growing through the slices were further transferred to rye agar (Lehtinen *et al.*, 2008). The isolates were maintained on rye agar at 6°C for 6–12 months before testing. Prior to testing, the isolates were multiplied on potato for one to three generations. In Sweden, agar plugs from cultures of *P. infestans* were transferred to tuber slices and incubated in Petri dishes. Sporangia formed on the slices were rinsed off with distilled water and the sporangial suspensions were used to inoculate leaflets of cv. Bintje. In Finland and Norway, sporangia were rinsed from rye agar in distilled water and used to inoculate potato leaflets (cv. Bintje). In all three cases, the leaflets were further incubated on wet filter paper in Petri dishes in natural daylight at 16°C. After 7 days' incubation, the sporangia formed on the leaflets were harvested in distilled water by rinsing (Sweden) or with a paintbrush (Finland and Norway). The sporangial density of the suspension was determined by calculating a mean of six haemocytometer counts and adjusted to 2×10^4 sporangia mL^{-1}.This suspension was used to inoculate potato leaflets for determination of latent period, growth rate and sporulation capacity. To determine frequency of infectious sporangia, an aliquot of the suspension was diluted to produce a suspension with 1×10^4 sporangia mL^{-1} and an aliquot of this suspension was further serially diluted four times by a factor of four to produce suspensions of 2500, 625, 156, and 39 sporangia mL^{-1}. The sporangial suspensions were chilled at 4°C for 2 h before inoculation to induce zoospore formation. Zoospore release was checked by microscope in the undiluted sporangial suspension.

Potato leaf production

Potato plants of cvs Bintje and Matilda were grown in the greenhouse. The cultivars were chosen on the basis of their susceptibility to late blight, cv. Bintje being susceptible and cv. Matilda moderately resistant. Pre-sprouted tubers were planted in 2-L pots and placed in the greenhouse with natural light supplemented with artificial light, a 16-h photoperiod and 15°C constant temperature. Fully expanded leaves from five upper leaf layers were collected from 6- to 7-week-old plants (Table 1).

Table 1 Schematic representation of the first replicate illustrating experimental design. Columns consisted of two plants, one for each potato cultivar (Bintje and Matilda). Numbers 1–40 in the cells represent the 40 *Phytophthora infestans* isolates. Isolate aggressiveness was tested for both potato cultivars simultaneously

		Columns															
		1		2		3		4		5		6		7		8	
Leaf position	Rows	Bintje	Matilda	Matilda	Bintje	Bintje	Matilda	Bintje	Matilda	Matilda	Bintje	Matilda	Bintje	Bintje	Matilda	Bintje	Matilda
Lowest leaf	1	18	18	12	12	16	16	23	23	37	37	11	11	3	3	27	27
Second leaf	2	14	14	19	19	10	10	21	21	13	13	9	9	38	38	17	17
Third leaf	3	2	2	26	26	4	4	33	33	34	34	20	20	24	24	40	40
Fourth leaf	4	15	15	28	28	30	30	36	36	31	31	5	5	7	7	39	39
Highest leaf	5	35	35	1	1	29	29	6	6	25	25	22	22	32	32	8	8

Experimental design

The aggressiveness of the isolates was evaluated using a resolvable row-column design (Williams *et al.*, 2002) with three (Norway, Sweden) or five (Finland) complete replicates. The row-column design enabled two-dimensional blocking. The row factor consisted of five different leaf levels: lowest leaf (1), second (2), third (3), fourth (4) and fifth (5) leaf upwards (Table 1). The column factor consisted of eight different test plants. As one to three whole columns were tested at the same time, variation between the columns also included variation between the test times. In addition, the experiment was arranged in order of the row-column design in growth cabinets (Norway and Sweden) or on the greenhouse bench (Finland) to take into account possible spatial variation in environmental conditions. In all countries, artificial light was used. In Sweden, the Petri dishes were stacked and illuminated from the side.

The measured aggressiveness components of the asexual disease cycle were frequency of infectious sporangia (FIS), length of latent period (LP), lesion growth rate (LGR) and sporulation capacity (SC). Aggressiveness was assessed on a cultivar with partial resistance to late blight as well as a susceptible one, because differences in aggressiveness among isolates have been reported to be greater on cultivars possessing partial resistance than on susceptible cultivars (Day & Shattock, 1997; Carlisle *et al.*, 2002).

Frequency of infectious sporangia (FIS)

FIS was determined on leaf discs. Fifty leaf discs, 14 mm in diameter, were cut with a cork borer from fully developed leaves of each cultivar for each isolate. Ten discs were placed, abaxial side up, in a Petri dish supplied with a filter paper wetted with distilled water. Altogether, five dishes were prepared for each isolate. Each dish was inoculated with one of the five sporangial dilutions: 100, 25, 6.25, 1.56 or 0.39 sporangia per disc, by placing a single 10-μL droplet at the centre of the leaf disc. In Finland, the lowest concentration was replaced by a suspension of 200 sporangia in 10 μL in replicates 2–5 because of lack of infections. The Petri dishes were sealed with Parafilm and placed in a growth chamber (Norway and Sweden) or on a greenhouse bench in natural light (Finland) at 15°C, according to the experimental design. The number of sporulating leaf discs per dish was assessed 7 or 9 days after inoculation on cvs Bintje and Matilda, respectively. FIS was calculated according to the most probable number (MPN) technique described in Halvorson & Ziegler (1933).

Detached leaflets

LP, LGR and SC were determined on detached leaflets at the same time as FIS. The same inoculation suspension was used for both leaf discs and leaflets. One experimental unit consisted of five detached leaflets of a single leaf. The leaflets were put abaxial side up in a plastic box (in Finland) or in five Petri dishes (each supplied with an irrigation mat or wetted filter paper, in Norway and Sweden, respectively). Each leaflet was inoculated with a single 10-μL droplet of 2×10^4 sporangia mL^{-1} (200 sporangia per leaflet) at the centre of the leaflet on the left side of the midrib. To avoid desiccation, the Petri dishes were sealed with Parafilm and the plastic boxes were covered with plastic. The leaflets were incubated together with the leaf discs under the same environmental conditions.

From day 3 after inoculation, the leaflets were checked every morning microscopically for sporulating lesions. In Norway and Sweden sporulation was also checked in the late afternoons or evenings. LP was determined as the time (in h) from inoculation to appearance of the sporangiophores on the lesion. Two diameters of the blight lesions were measured in millimetres at a 90° angle for three consecutive mornings starting on day 5 (cv. Bintje) or day 7 after inoculation (cv. Matilda). The first diameter was measured along the midrib. Lesion size was determined by using the formula for an area of ellipse: $A = a * b * \pi$, where a and b were the shortest and longest radii of the ellipse, respectively. The two radii were calculated by dividing the measured diameters of the lesion by two. The estimation of average lesion growth rates (mm day^{-1}) for the isolates was based on the average radius $[(a + b)/2]$ of each lesion.

The number of sporangia produced by each lesion was determined 7 or 9 days after inoculation on cvs Bintje and Matilda, respectively. In Finland, the leaflets were transferred into 5 mL of physiological saline solution (Sysmex

Table 2 Number of *Phytophthora infestans* isolates tested, total number of potato leaflets used for testing per cultivar and percentage of sporulating leaflets for both potato cultivars (Bintje and Matilda) in each country

Isolates	No. of isolates tested	No. of leaflets tested per cultivar	Percentage of sporulating leaflets	
			Bintje	Matilda
Finnish	25	625	69	57
Danish	15	375	66	62
Swedish	23	345	71	54
Danish	15	225	72	54
Norwegian	25	375	92	49
Danish	15	225	76	47

Europe Gmbh) after lesion size had been measured. In Norway, leaflets with lesions were transferred to distilled water or 70% ethanol when it was not possible to determine the sporangial concentration the same day. In Sweden, leaflets were always put into 70% ethanol and stored at 4°C until spore counting. The liquid with the leaflets was vortexed for 30 s and the number of sporangia produced on the lesion was determined by counting in a haemocytometer as described above. SC was calculated by dividing the number of sporangia produced on the lesion by the final lesion area.

Data analysed

Percentages of sporulating leaflets for both cultivars (Bintje and Matilda) in each country are presented in Table 2. Means of the measurements for the sporulating leaflets of each leaf were used as observations in the statistical analyses. Consequently, the number of replicates for an isolate was lower than the initial number when none of the five leaflets of a leaf produced sporangiophores during the observation period. In the Norwegian data for cv. Matilda, seven isolates did not sporulate on any of the leaflets of the three replicates and were thus excluded from the statistical analyses. For the same reason, two isolates on cv. Bintje and seven isolates on cv. Matilda were excluded from the Swedish data, and two isolates were not tested at all because of insufficient sporulation on potato leaflets.

Statistical analyses

The data of the two cultivars and the three laboratories were analysed separately. The statistical analyses of the FIS, LP, and SC were based on the following model for a resolvable row-column design:

$$y_{ijkl} = \mu + \rho_i + \alpha_{j(i)} + \beta_{k(i)} + I_l + \varepsilon_{ijkl}, \quad (1)$$

where y_{ijkl} was the observation for the lth isolate in the kth column and the jth row within the ith replicate of the design; μ was a parameter for the overall mean; ρ_i was the random effect for the ith replicate, and $\alpha_{j(i)}$ and $\beta_{k(i)}$ were the random effects for the jth row and the kth column within the ith replicate, respectively; I_l was the fixed effect for the lth isolate and ε_{ijkl} the experimental error. The random effects ρ_i, $\alpha_{j(i)}$, $\beta_{k(i)}$, and ε_{ijkl} were assumed to be independent, and within each term the effects were assumed to be independent and normally distributed with zero means and variances σ^2_ρ, σ^2_α, σ^2_β, σ^2_ε, respectively. According to the model, the observations y_{ijkl} are normally distributed with mean $E(y_{ijkl}) = \mu + I_l$ and variance $\mathrm{Var}(y_{ijkl}) = \sigma^2_\rho + \sigma^2_\alpha + \sigma^2_\beta + \sigma^2_\varepsilon$. To satisfy the assumptions of the constancy of the variance for all observations and normality of the data, transformations to the values of the response variables were needed. The FIS and the SC were square-root-transformed. LP was analysed on the original scale with the exception of Bintje data in Norway, for which the reciprocal ($1/y$) transformation was applied. Mean estimates for the isolates [estimates of $E(y_{ijkl})$] were obtained by substituting the estimates for μ and I_l from the statistical analyses. The accuracies of the estimated means were expressed through 95% confidence intervals. When transformations were used, the estimated means and the lower and upper limits of the confidence intervals were back-transformed to the original scale. The comparisons between the isolate means of the countries were made by two-sided t-type tests.

The estimation of average LGRs for the isolates was based on average radii of the lesions measured during 3 days from day 5 and 7 onward on cvs Bintje and Matilda, respectively. The number of repeated measurements for each isolate in the replicates ranged between one and three depending on lesion appearance. The relationship between the average radius and the measuring time t_m was modelled by a straight line by allowing different intercepts and slopes for each isolate. The mean portion of the statistical model employed was thus: $E(y_{ijklm}) = a_l + b_l t_m$, where y_{ijklm} was the mth repeated measurement of the average radius of lesion for the lth isolate in the kth column and the jth row within the ith replicate, and slope b_l indicating the average LGR for isolate l. The measurements of the same leaf over time tended to be intercorrelated, which was taken into account with the covariance structure of the repeated measurements. The 'best' covariance structures were chosen through a comparison of different models by the likelihood ratio test and information criteria (Littell *et al*., 2006). In analyses of the Swedish data for cv. Bintje and the Norwegian data for cv. Matilda, the 'unstructured' covariance pattern appeared most appropriate. In this structure, variances and pairwise covariances of observations at various measuring times are allowed to be unequal. In the analysis of the Finnish data for cv. Matilda, an adequate covariance structure proved to be the first-order autoregressive structure [AR(1)], in which variances are homogeneous but pairwise covariances decrease toward zero with increasing length of time interval between measurements. For all the other data the heterogeneous AR(1) structure was chosen to accommodate unequal variances of observations at different measuring times. Besides this within-leaf portion of the covariance structure, the model

included the between-leaf portion induced by the experimental design, i.e. covariance caused by random effects for replicates, rows and columns. After finding a satisfactory covariance structure for each dataset, mean LGRs for the isolates of the different countries were estimated and compared by two-sided *t*-type tests.

All models were fitted by using the restricted maximum likelihood (REML) approach. The method detailed by Kenward & Roger (1997) was used to calculate degrees of freedom. The appropriateness of the models was examined through graphic analysis of residuals. The normality assumption of the residuals was checked using the box plot (Tukey, 1977), and the residuals were also plotted against the fitted values. The plots showed that the chosen models fitted the data adequately, with the exception of a few outliers whose influence on the results was examined by deleting them from the data one at a time. Since their influence was minor, results based on all data are presented. The analyses were performed by the MIXED procedure in SAS/STAT software (Littell *et al*., 2006).

Results

The differences in aggressiveness of *P. infestans* between the Nordic countries were generally small or inconsistent between the cultivars. Because of marked variation in results between the three laboratories, the differences in aggressiveness of the *P. infestans* populations from the different countries were compared indirectly via the Danish isolates, which were divided between the test laboratories. There were no clear groups of isolates assessed as aggressive or weak on all aggressiveness components, apart from the few Swedish and Norwegian isolates unable to cause infections on the detached leaflets.

Frequency of infectious sporangia (FIS)

Estimated mean FIS values were less than 1% in the data from the different countries, with the exception of the results for Norwegian isolates on cv. Bintje, where the mean estimate was 2.7% (Fig. 1). However, this was probably caused by laboratory effects, since the mean estimate for the Danish isolates tested in Norway was similar in magnitude and clearly higher than in the other countries (Table 3a). Furthermore, the results from cv. Bintje in Norway were not consistent with those from cv. Matilda. The data from cv. Matilda included the greatest amount of low infection frequencies: 17 of the 40 isolates (43%) had infection frequencies of less than 1% in all three replicates. In addition, the proportion of sporulating leaflets was only 49% on cv. Matilda in contrast to 92% on cv. Bintje (Table 2). Because of the excessive number of values between 0 and 1%, the Norwegian data for cv. Matilda were not modelled.

Length of latent period (LP)

On cv. Bintje, the differences in mean LPs between countries were minor within the test laboratories (Table 3b, Fig. 2). In cv. Matilda, a tendency towards shorter LPs among Danish isolates was observed in all laboratories, the difference being statistically significant in Norway and Finland. However, the estimated mean differences were 14 h for Norway and 7.7 h for Finland, while the time interval between observations was 24 h in Finland and in Norway readings were taken twice a day. Comparison of the means of the Danish isolates in the three laboratories indicated that the differences in LP between the Finnish, Swedish and Norwegian isolates were mostly caused by the laboratory effect for both cultivars.

Lesion growth rate (LGR)

Regarding mean LGRs, the differences between countries on cv. Bintje were again minor within the test laboratories, but on cv. Matilda there was some evidence that the Danish isolates differed from the rest (Table 3c, Fig. 3). The estimated mean LGR of the Danish isolates was 0.6 and 0.8 mm day^{-1} greater than those of the Swedish and Norwegian isolates, respectively, but 0.5 mm day^{-1} less than that of the Finnish isolates. However, differences were smaller than the accuracy of the measurements, which was 1 mm. The apparent laboratory effect hampered the comparison of the Finnish, Swedish and Norwegian isolates.

Sporulation capacity (SC)

Differences in SC between the Nordic *P. infestans* populations were generally minor after taking into account a considerable laboratory effect (Table 3d, Fig. 4). The Danish isolates seemed to produce more spores than Norwegian isolates on both cultivars and they seemed to produce more spores than Swedish isolates on cv. Bintje. However, the evidence for true differences between these countries is not convincing because of inconsistencies between the results on the two cultivars (Table 3d).

Effects of blocking factors (replicate, row and column factor) on the variability of observations

In the statistical analyses the magnitude of the variability of individual observations could be divided into four components: variance between replicates (σ^2_ρ), variances between rows (σ^2_α) and between columns (σ^2_β) within replicates, and error variance (σ^2_ε). After the four variance components had been estimated, the percentage of the total variability explained by each source of variation was computed as the ratio of the variance component estimate to the variance component total, multiplied by 100. The percentages show the relative significance of each blocking factor for the overall variability of the observations (Table 4). The column factor caused considerable variation in Finland, while effects of replicate and row factor (leaf position) were generally small. Unlike in Finland, there was no dominating source of variation in Norway and Sweden. The effect of row factor on the variance of observations was mainly small in each laboratory. In spite of careful

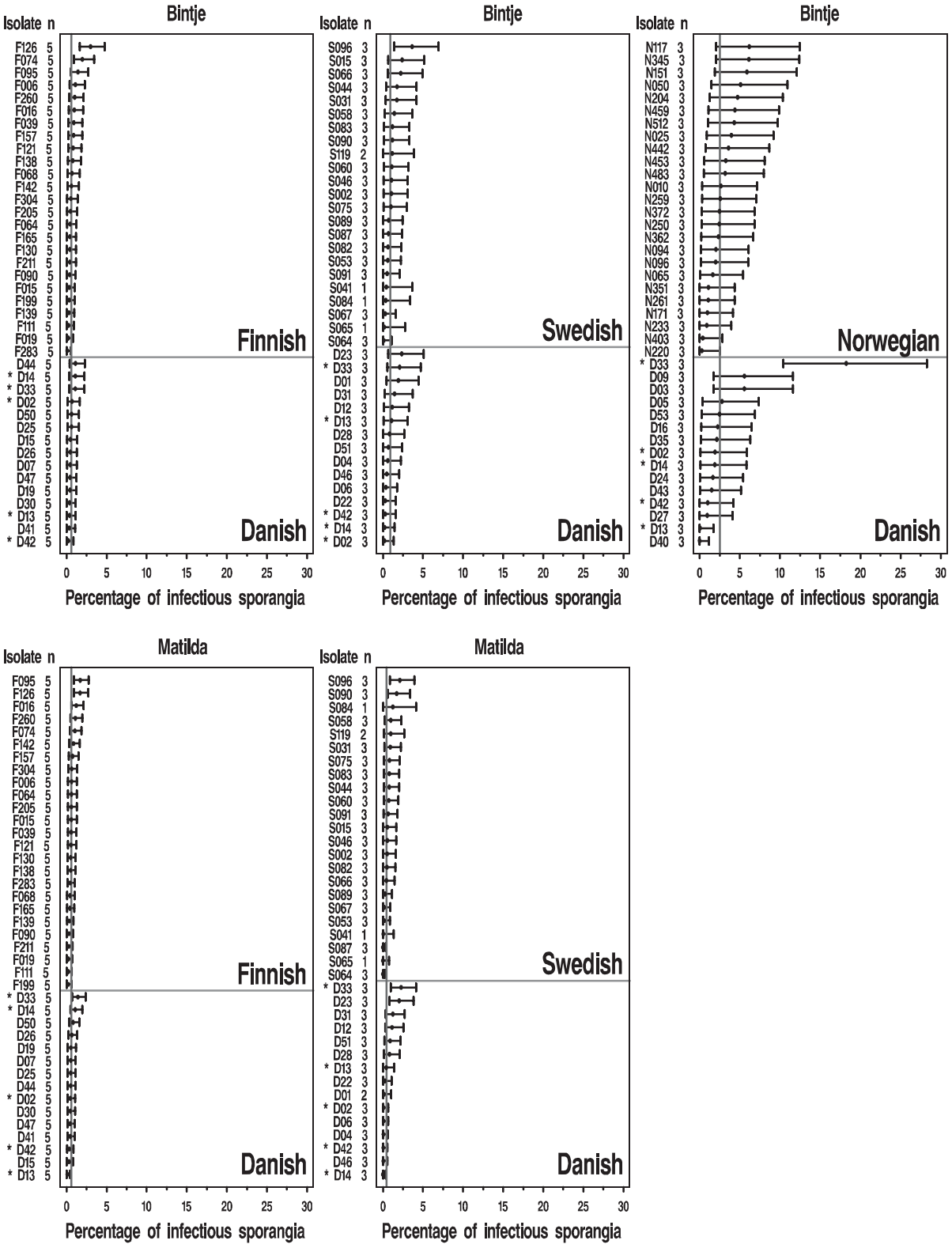

Figure 1 Estimated mean percentages of sporangia capable of infecting potato leaf discs and 95% confidence intervals for the means of *Phytophthora infestans* isolates collected from four Nordic countries and tested in Finland (left), Sweden (centre) and Norway (right). Estimated means of all the isolates within each laboratory are illustrated by vertical lines. The Danish isolates which were tested in all three laboratories are marked with asterisks. n = number of replicates.

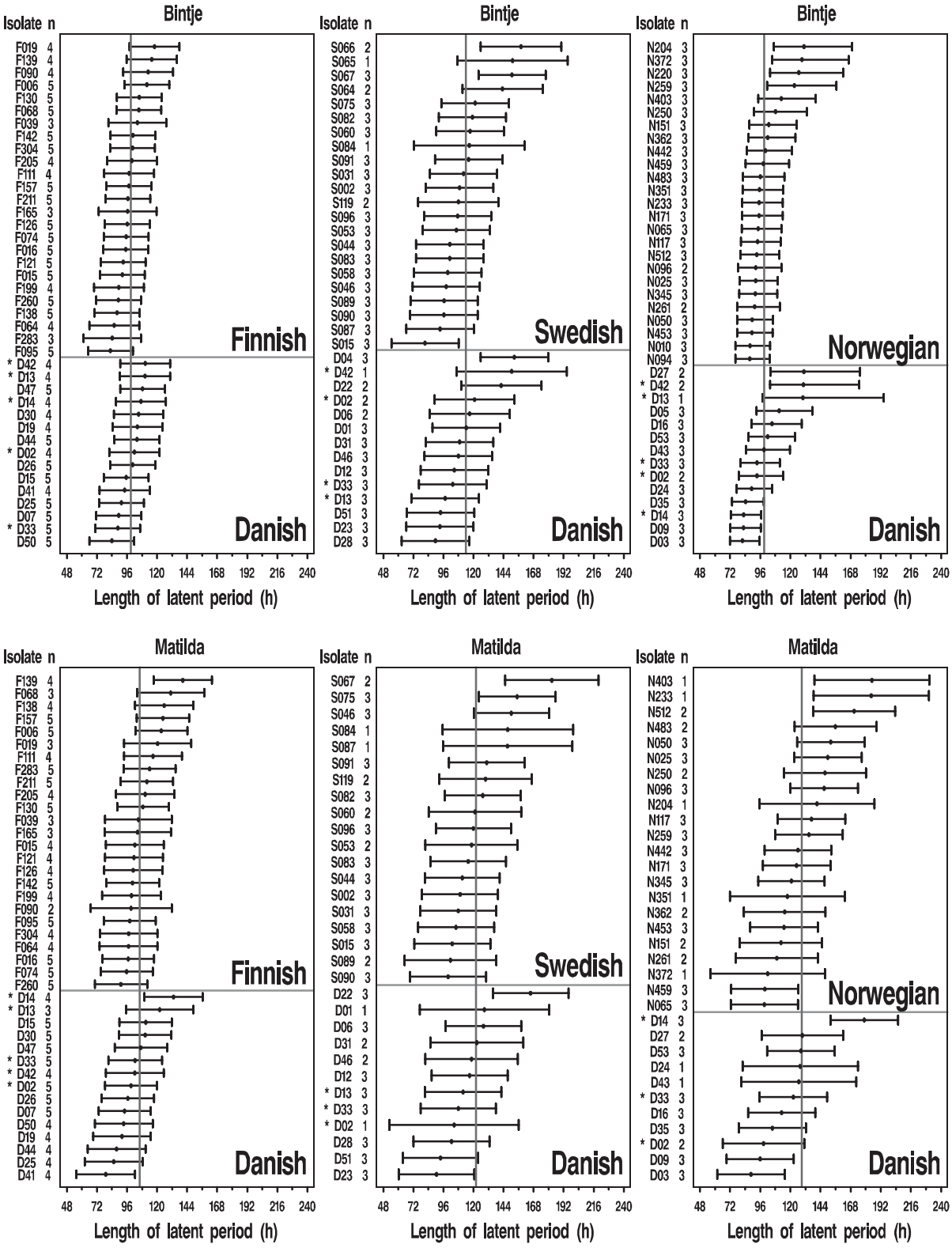

Figure 2 Estimated mean length of latent periods and 95% confidence intervals for the means of *Phytophthora infestans* isolates collected from four Nordic countries and tested in Finland (left), Sweden (centre) and Norway (right). Estimated means of all the isolates within each laboratory are illustrated by vertical lines. The Danish isolates which were tested in all three laboratories are marked with asterisks. n = number of replicates.

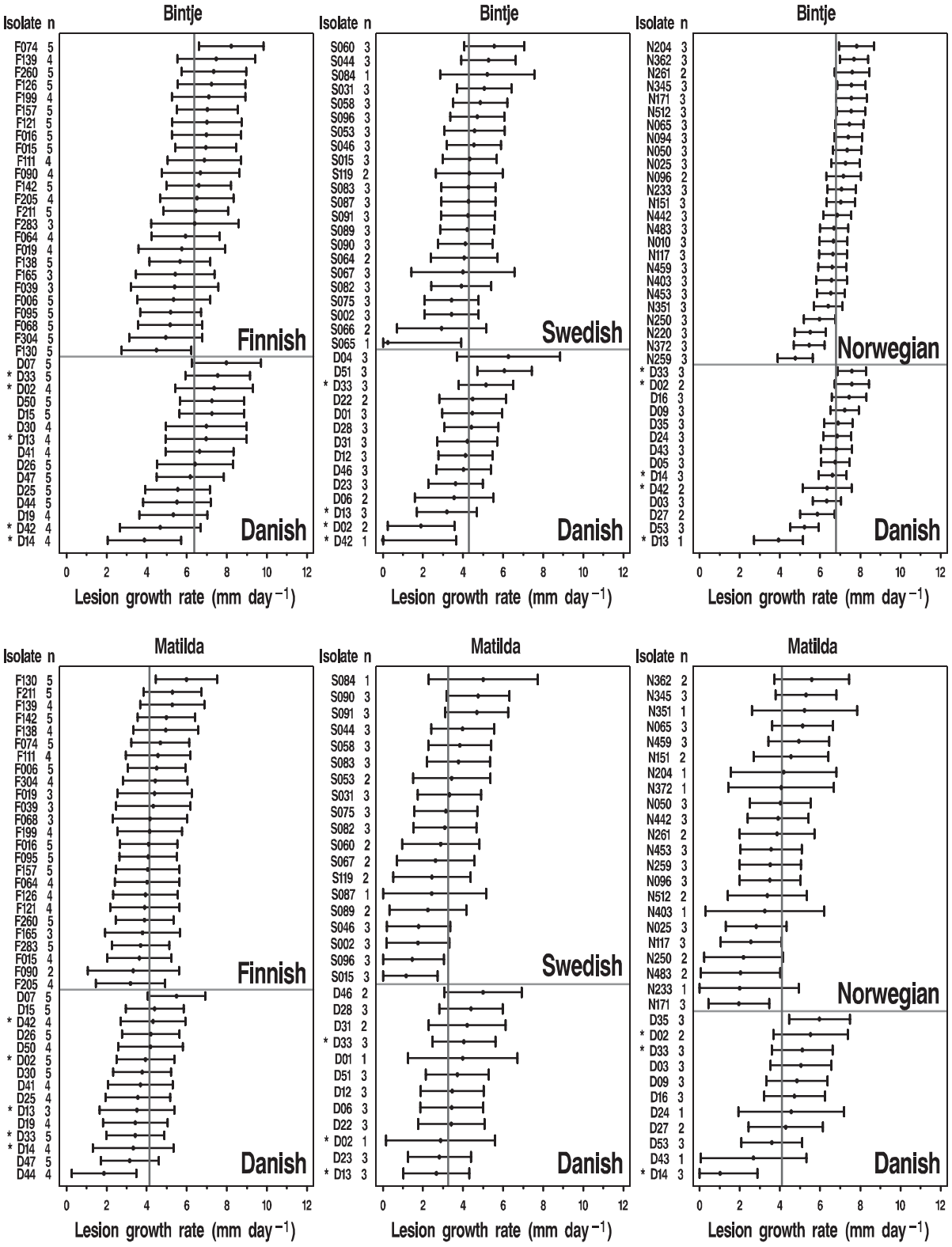

Figure 3 Estimated mean lesion growth rates (LGRs) and 95% confidence intervals for the means of *Phytophthora infestans* isolates collected from four Nordic countries. and tested in Finland (left), Sweden (centre) and Norway (right). Estimated means of all the isolates within each laboratory are illustrated by vertical lines. The Danish isolates which were tested in all three laboratories are marked with asterisks. n = number of replicates.

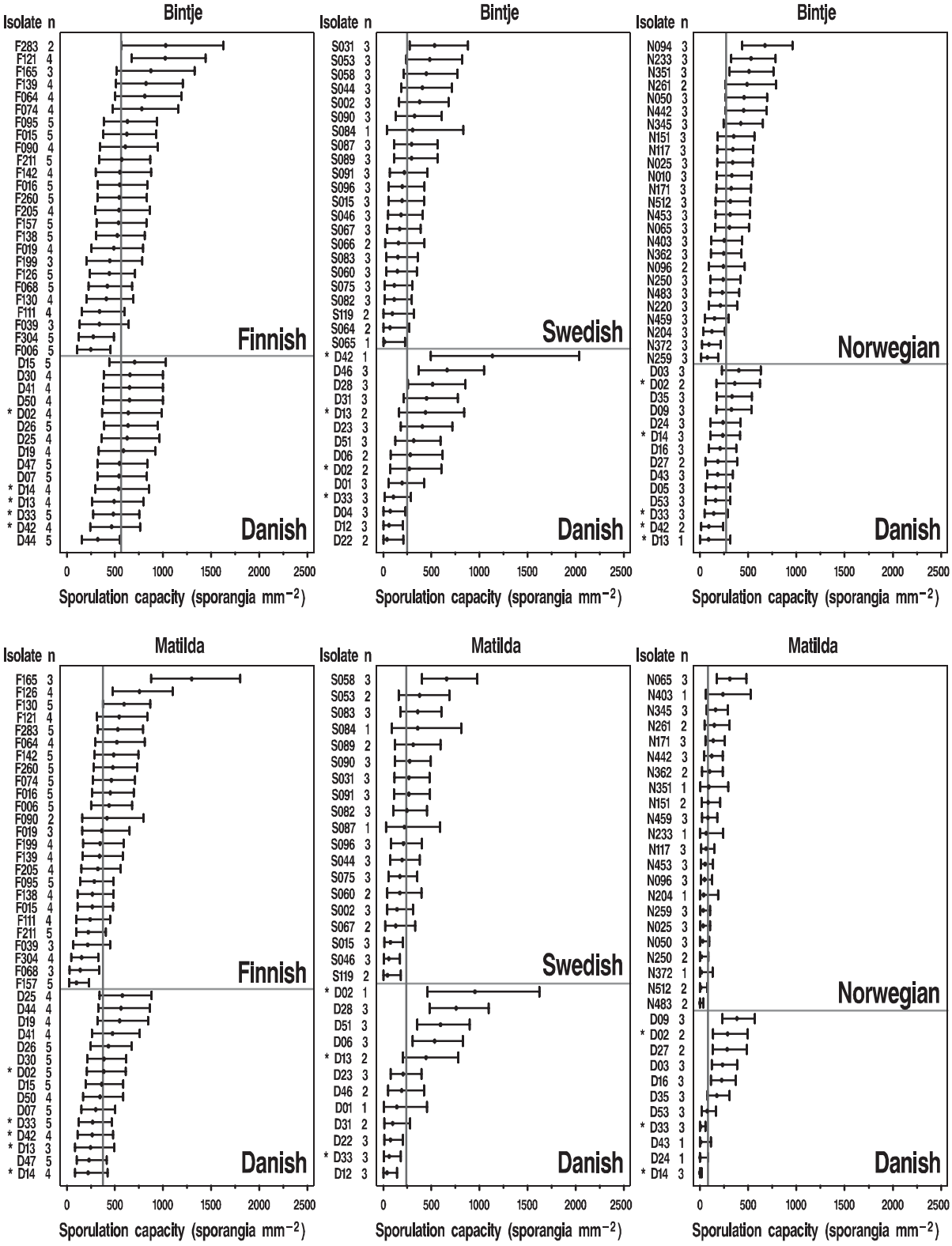

Figure 4 Estimated mean sporulation capacities and 95% confidence intervals for the means of *Phytophthora infestans* isolates collected from four Nordic countries and tested in Finland (left), Sweden (centre) and Norway (right). Estimated means of all the isolates within each laboratory are illustrated by vertical lines. The Danish isolates which were tested in all three laboratories are marked with asterisks. n = number of replicates.

Table 3 Comparison of (a) frequency of infectious sprorangia (FIS; %), (b) length of latent period (LP; h), (c) lesion growth rate (LGR; mm day^{-1}) and (d) sporulation capacity (SC; sporangia mm^{-2}) of *Phytophthora infestans* on potato cvs Bintje and Matilda

	Bintje				Matilda			
Origin of isolates	No. of isolates	*n*	Mean (and 95% CI)	*P*-value	No. of isolates	*n*	Mean (and 95% CI)	*P*-value
(a) Frequency of infectious sporangia								
Finnish	25	125	0.7 (0.4–1.0)	0.39	25	125	0.6 (0.4–0.9)	0.39
Danish	15	75	0.6 (0.3–0.9)		15	75	0.5 (0.3–0.8)	
Swedish	23	62	1.0 (0.6–1.4)	0.49	23	62	0.5 (0.2–0.9)	0.53
Danish	15	45	0.8 (0.5–1.4)		15	44	0.4 (0.2–0.8)	
Norwegian	25	75	2.7 (2.0–3.5)	0.51	25	75	[a]	
Danish	15	45	2.3 (1.5–3.2)		15	45		
(b) Length of latent period								
Finnish	25	112	98 (91–106)	0.62	25	104	109 (101–116)	0.04
Danish	15	68	100 (92–108)		15	66	101 (93–109)	
Swedish	22	59	114 (92–135)	0.97	19	48	125 (108–143)	0.14
Danish	14	37	114 (94–134)		12	30	116 (100–132)	
Norwegian	25	73	99 (83–123)	0.68	22	50	134 (117–151)	0.02
Danish	14	37	98 (84–119)		11	27	120 (104–135)	
(c) Lesion growth rate								
Finnish	25	305	6.4 (6.0–6.7)	0.88	25	307	4.3 (4.0–4.7)	0.04
Danish	15	181	6.4 (6.0–6.9)		15	195	3.8 (3.4–4.2)	
Swedish	22	167	4.3 (4.0–4.7)	0.61	19	144	3.0 (2.6–3.4)	0.06
Danish	14	102	4.2 (3.8–4.6)		22	88	3.7 (3.1–4.2)	
Norwegian	25	210	6.9 (6.7–7.1)	0.23	22	145	3.9 (3.4–4.3)	0.03
Danish	14	106	6.7 (6.4–6.9)		11	78	4.6 (4.1–5.2)	
(d) Sporulation capacity								
Finnish	25	106	559 (411–731)	0.89	25	104	381 (276–503)	0.62
Danish	15	66	565 (416–737)		15	66	365 (263–484)	
Swedish	22	59	219 (133–325)	0.05	19	48	219 (119–350)	0.12
Danish	14	36	300 (204–413)		12	29	277 (173–406)	
Norwegian	25	73	304 (172–474)	<0.001	22	50	70 (48–98)	0.05
Danish	14	37	213 (118–336)		11	27	107 (73–146)	

CI = confidence interval of the mean, *n* = number of observations.

[a]The Norwegian data for cv. Matilda were not modelled because of the excessive number of values between 0 and 1%.

blocking and other precautions, typically more than half of the total variation was attributable to experimental error.

Discussion

Differences in aggressiveness components between countries were generally limited or not in agreement between the cultivars. Although there were statistically significant differences in the analyses of LP, LGR and SC, the differences were smaller than the accuracy of the measurements and apparently insignificant for epidemic development. Thus, there is no evidence that different values of aggressiveness components of DSS submodels are needed for each Nordic country.

Despite the lack of differences between countries, it is possible that there are significant differences between the regions within the countries. This is suggested by the variation between the isolates, and the fact that regions with different types of potato production are separated by hundreds of kilometres. Because of the small sample size, it was not possible to study regional differences in *P. infestans* populations within the countries. Flier & Turkensteen (1999) found statistically significant differences in LP between three potato-growing areas in the Netherlands. The LP was longer in an allotment garden complex than in commercial potato production, probably because of the presence of isolates adapted to tomato. However, poor blight management and lack of rotation are general characteristics of allotments and gardens where oospore-derived epidemics could be more common than in commercial potato-growing areas (Zwankhuizen *et al*., 2000).

Table 4 Percentages of total variability of observations associated with replicates, rows and columns of the experimental designs [(variance component estimate/variance component total) × 100] for frequency of infectious sporangia (FIS), length of the latent period (LP), lesion growth rate (LGR)and sporulation capacity (SC) of *Phytophthora infestans* on potato cultivars Bintje and Matilda. For LGR the percentages of the last measurement time are presented

	Finland								Norway								Sweden							
	Bintje				Matilda				Bintje				Matilda				Bintje				Matilda			
Source of variation	FIS	LP	LGR	SC	FIS	LP	LGR	SC	FIS	LP	LGR	SC	FIS	LP	LGR	SC	FIS	LP	LGR	SC	FIS	LP	LGR	SC
Replication	4	2	4	8	7	0	4	2	0	32	19	8	[a]	7	2	0	0	15	2	4	1	6	0	11
Row	1	4	0	0	0	0	19	6	0	0	12	14	[a]	0	1	18	4	0	0	4	8	7	10	0
Column	9	27	20	42	17	47	23	42	6	5	2	30	[a]	14	0	0	8	7	12	0	13	6	7	1
Residual	86	66	76	49	76	53	54	48	93	64	67	49	[a]	79	97	82	87	78	87	92	77	81	84	88

[a]The Norwegian data for cv. Matilda were not modelled because of the excessive number of values between 0 and 1%.

The *P. infestans* populations in the Nordic countries have been shown to be genetically diverse and it is suggested that this variation is maintained by sexual reproduction (Brurberg *et al*., 1999). Potential for oospore-derived infections was high in the Nordic countries in the present study, as both mating types were present in 29–56% of the fields where more than one isolate was tested (Lehtinen *et al*., 2008). Suspected oospore-derived epidemics have been observed in Sweden (Andersson *et al*., 1998; Widmark *et al*., 2007) and Finland (Lehtinen & Hannukkala, 2004). Mayton *et al*. (2000) showed that isolates derived from oospores generally tended to be less aggressive than clonal isolates. During the asexual disease cycle, genotypes possessing the most favourable allele combinations accumulate in the *P. infestans* population by selection. During mating, favourable sets of alleles break up and new sets are randomly formed (Barton & Charlesworth, 1998). Therefore, oospore-derived progeny should be generally less aggressive than heavily selected clones. The isolates in this study were collected early in the season, leaving little time for natural selection. This may partly explain the large variation in aggressiveness and why some of the isolates sporulated so weakly. However, by combining sexual recombination with an effective clonal propagation and spread, *P. infestans* has the ability to respond quickly to selection pressure and successful isolates can, over short periods, become dominant in the pathogen populations. If the isolates had been collected late in the season, it is likely that the levels of aggressiveness would have been higher.

High variation in results between the test laboratories hampers comparisons of the results of this study to other groups' results and emphasizes the need for caution when making such comparisons. Many groups have used the highly susceptible cv. Bintje in aggressiveness tests, but nobody has used cv. Matilda. Therefore, only results for cv. Bintje were compared. The LP of Nordic isolates (mean 104 h, range 83–135 h) was in line with the results of Flier & Turkensteen (1999) (81–144 h), but clearly longer and more variable than among isolates collected from Northern Ireland (3–4 days) (Carlisle *et al*., 2002). The LP was extremely short (43 h) among isolates collected from France and Switzerland (Knapova & Gisi, 2002), but this was probably mainly an effect of the higher incubation temperature (20°C) used in these tests, as LP is highly dependent on temperature (Andrade-Piedra *et al*., 2005). Also, the percentage of sporulating leaflets was clearly lower among Nordic (excluding Norwegian isolates) and Dutch (Flier & Turkensteen, 1999) isolates of *P. infestans* than in Northern Ireland (Carlisle *et al*., 2002) and France (Lebreton *et al*., 1999). The percentage of sporulating leaflets was similar between Norwegian and French isolates.

The SC of Nordic isolates was somewhat higher than in France and Switzerland (Lebreton *et al*., 1999; Knapova & Gisi, 2002; Montarry *et al*., 2006), but less than 1% of the SC of Northern Ireland isolates (Carlisle *et al*., 2002). However, measurement of SC seems to be quite sensitive to test conditions. In Finland, the mean SCs of all the isolates were the highest for both cultivars, approximately twice as high as in Norwegian and Swedish tests on cv. Bintje. As temperature and relative humidity were kept as similar as possible, the difference probably resulted from different light conditions. In Norway and Sweden, the tests were carried out under artificial light in growth chambers, while in Finland natural light in a greenhouse was supplemented with artificial light when needed. In Northern Ireland, the tests were also carried out in natural light (Carlisle *et al*., 2002). In Norway, the tests were carried out during the first half of the year, in Sweden during the second half of the year and in Finland the first replicate was started in May and the last finished in October. The different timing of the tests in different countries might have caused some of the observed variation between the countries, since SC has been shown to vary through the year (Sujkowski, 1986a,b). In fact, the test time affected the LP and LGR results of cv. Bintje considerably in Norway: towards the spring the estimated LP decreased from 107 h (first replicate) to 92 h (third replicate) and, similarly, the estimated LGR increased from 0.6 to 2.5 mm day^{-1}. Concluding the discussion above, the Nordic *P. infestans* population appears to be less or equally aggressive in

terms of LP, percentage of sporulating leaflets and SC compared to other studied European populations of *P. infestans*. However, the population in Northern Ireland seems much more aggressive than Nordic *P. infestans* populations, probably because of more heavily selected clones in the absence of recombination.

As far as the authors are aware, FIS has not been assessed in other studies by the method used in this study. The method was adopted from bacteriology, where it is known as the most probable number (MPN) technique, but it has also been used in mycological studies to count viable spores (Halvorson & Ziegler, 1933). Multiplication of the values of FIS and SC results in a variable representing the number of infectious sporangia per mm^2 of blight lesion. This variable is superior to SC from an epidemiological point of view, as it does not include sporangia unable to cause infections and thus incapable of promoting epidemic development. Unfortunately, FIS values were extremely low in the present study. However, sporangia harvested directly from the field in Denmark showed a considerably higher FIS than in this study (B. J. Nielsen, unpublished). Therefore, it is probable that the low values reported here do not represent the field aggressiveness of the isolates, but are an artefact of the test method. Also, using leaf discs instead of leaves might result in a low infection rate because of a plant reaction triggered by the cutting. No attempt was made to construct an aggressiveness index because of lack of coefficients by which each aggressiveness component of the index should be weighted (Lebreton *et al*., 1999; Carlisle *et al*., 2002).

Day & Shattock (1997) and Carlisle *et al*. (2002) found more marked differences between isolates in aggressiveness on cultivars exhibiting a higher level of race-nonspecific resistance. However, Montarry *et al*. (2006) found larger differences in aggressiveness on cv. Bintje than on the partially resistant cv. Désirée in French *P. infestans* populations. Findings in the Nordic populations support the fact that susceptible cultivars might be more discriminant in some populations, but not in others. Moreover, some of the isolates differed strikingly in aggressiveness on the two cultivars tested. Therefore, use of several cultivars with different levels of race-nonspecific resistance is desirable as it increases the representativeness of the results.

In variety trials, cv. Matilda has proved to possess relatively high partial blight-resistance, while cv. Bintje is fully susceptible. The presumed higher resistance of cv. Matilda over cv. Bintje was evident in LGR, but to a lesser extent in SC. Essential differences between the cultivars were not observed in FIS and LP, except in the Norwegian data. In Norway, the mean LP was 35 h longer and FIS smaller in cv. Matilda than in cv. Bintje. The results were similar between the Norwegian and Danish isolates, suggesting that the differences in results between the countries were caused by differences between laboratories. Actual differences between the cultivars in resistance to late blight can vary considerably from the results presented here because race-nonspecific resistance includes many more components than those assessed in the present study.

High experimental variation is a well-known feature of these kinds of tests. Therefore, it is important to test all isolates in the same environmental conditions, and pay strict attention to inoculum and test-plant production and preparation. Yet, even under a tightly controlled environment, experimental variation often remains high (Harrison *et al*., 1994). In addition to controlling the test conditions, the experimental variation can also be controlled by selecting an appropriate experimental design with adequate blocking. Despite extensive blocking, the residual variation here remained high. Differences between replicates were probably caused by the time of year in which the tests of the replicate were carried out, as discussed above. It was impossible to separate whether the considerable variation observed in the column factor in Finland was caused by different test times, different test plants or both. In an earlier study in Finland, variation in results within the test day was much greater between inocula prepared from different leaves than within replicates from one inoculum (A. Lehtinen, unpublished). Therefore, most of the variation between columns was probably caused by inoculum production and preparation. Inoculum can differ between tests because of different ages of sporangia or differences in inoculum preparation, e.g. length of time kept at +4°C, and extent of subsequent mixing of zoospore suspension. The larger variation in the column factor in Finland than in Norway and Sweden was probably caused by the fact that several different workers carried out the tests in Finland.

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