# Differential Host-Pathogen Interactions Among Clones of Poplar and Strains of *Xanthomonas populi* pv. *populi*

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## ABSTRACT

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To verify the occurrence of physiologic races in *Xanthomonas populi* pv. *populi*, the causal agent of oozing canker of poplar, the virulence of 19 strains was compared by inoculating five poplar clones and one willow clone in two sets of tests performed during June and September, respectively. Canker severity was measured 2 yr later by determining the lengths and the girdling index of cankers. Analysis of variance indicated a significant poplar clone–strain interaction and no significant clone–strain–inoculation date interaction for most strains, using the two variables. Five putative physiologic races were characterized. Race 3 was totally avirulent on the clone Italica, although the other races gave relatively small differences in canker severity. Strains belonging to races 1, 2, 3, and 5 were isolated in continental Europe (Belgium, France, and the Netherlands), whereas race 4, more virulent to *Populus trichocarpa* clones, was isolated in Britain or close to the Belgian coast.

*Additional keywords*: disease resistance, poplar breeding.

Bacterial or oozing canker of poplar (*Populus* spp.) is a serious disease in northern Europe caused by *Xanthomonas populi* pv. *populi* (formerly called *Aplanobacterium populi* or *Aplanobacter populi*) (24,25,27). Typical symptoms include long cracks on stems or branches surrounded by swollen and irregular lips that ooze a profuse, viscous, and whitish slime during the spring. These cankers may entirely girdle a stem, causing the upper part to die back, or may be more limited in extension. *X. p. populi* is

specific to all tested species of the genus *Populus*, whereas a related taxon, *X. p.* pv. *salicis* causes oozing canker on willow species (6,7). Poplars are fast-growing trees widely used in Europe as logs for sawtimber, pallets, packing cases, paper pulp, veneer for plywood, packing boxes, or matches and more recently as chips for fuelwood. There is a long tradition of poplar cultivation in European countries, and large areas are planted with highly selected clones. The main species breeded include *P. nigra* L., *P. deltoides* J. Bartrem ex Marsh, *P. trichocarpa,* Torr. & A. Gray, and their hybrids *P.* × *euramericana* (Dode) Guinier and *P.* × *interamericana* in the interfertile sections *Aigeros* and *Tacamahaca*, and *P. tremula* L., *P. alba* L., and *P. tremuloides* Michx. and their hybrids in the *Leuce* section. The susceptibility of numerous other poplar species also have been investigated (31). On the 250,000 ha planted with poplar in France, about 100,000 ha are planted in the endemic zone of *X. populi* and can be naturally contaminated. Cankers on susceptible clones may seriously devalue the quality of the poplar wood, especially for fiber and veneer purposes. Furthermore, the planting of thousands of susceptible poplars, like the clones Blanc du Poitou in northern France, Rap in the Netherlands, and Mühle-Larson in Germany, has led to increased canker outbreaks. The disease spread out of its natural area, especially toward the region north of the Waal and Rhine rivers, where climatic conditions were favorable to the bacterium. Thus, it is important to know whether the newly selected poplar clones proposed to poplar growers are susceptible to this disease.

Susceptibility of poplar clones is determined by artificial inoculation with a bacterial cell suspension from natural slimes (15,17,34) or from pure cultures (1,9,24,30). Since 1965, all the progeny and new crosses have been routinely subjected to this test, representing more than 1,250,000 inoculations to date. For instance, not one case of natural infection by *X. populi* has been recorded in the series of 10 Unal clones in over 20 yr, confirming the reliability of this selection test, which is a unique example in the field of resistance breeding with forest species (31). However, because screening of poplar selections for resistance has been done with one standard strain (e.g., SPm in France and M1 in Belgium), it is possible that poplars resistant to standard strains are susceptible to strains of a different race. This could lead to new canker outbreaks if susceptible poplars are planted close to the foci of this race. For this reason, several surveys were carried out to verify the virulence of *X. p. populi* strains (27–29) that showed a wide range of aggressiveness among strains and suggested a differential compatibility between hosts and pathogens. Contrary to what has been described for the flax-rust model (11), however, resistance in bacterial canker of poplar is more likely partial than complete, and intermediate reactions are generally more difficult to classify and more dependent on environmental effects and, thus, need more precise and analytical tests (10).

Differential virulence has been reported for various host-specific pathogenic bacteria. Several races or virulence groups have been described in *Pseudomonas syringae* pv. *glycinae* (3,32), *X. campestris* pv. *malvacearum* (14), *X. c.* pv. *vesicatoria* (2), *X. c.* pv. *oryzae* (13,21), and *Erwinia amylovora* (23). Bacterial groups have been designated as "virulence groups" on the grounds that disease severity in cultivar–strain experiments was variable; however, Mew (20) indicated that race is the proper term to designate the bacterial groups. In this paper, we present evidence for the occurrence of races in *X. p. populi* by analyzing the severity of canker lesions formed by 19 bacterial strains on five poplar clones. In addition, we verified that all the races belong strictly to *X. p. populi* and not to *X. p. salicis*.

## MATERIALS AND METHODS

**Bacterial strains and plant material.** Strains of *X. p. populi* and *X. p. salicis* used for inoculation originated from various areas in Europe and were isolated from several poplar species (Table 1). Strains and clones were chosen according to the results of previous surveys (27,28). Most of the strains were isolated

TABLE 1. Pathovars of *Xanthomonas populi* used to test host–pathogen interactions in poplar (*Populus* spp.) and willow clones (*Salix* sp.)

| Strain and race | Original designation | Origin | |
|---|---|---|---|
| | | Host[a] | Location |
| *X. p.* pv. *populi* | | | |
| Race 1[b] | | | |
| 2787[c] | SPm18 | *P.* × *euramericana* Blanc du Poitou | Oise (France |
| 2221 | Brugelette | *P.* × *euramericana* | Hainault (Belgium) |
| 2210 | Maffle | *P.* × *euramericana* Regenerata | Hainault (Belgium) |
| 2181 | S.387-76 | *P. deltoides* × *P. trichocarpa* S.387-76 | Flandre Orientale (Belgium) |
| Race 2 | | | |
| 2220 | S.57 | *P. deltoides* × *P. trichocarpa* S.5-7 | Flandre Orientale (Belgium) |
| 2219 | McKee | *P. d. angulata* × *P. trichocarpa* | Hainault (Belgium) |
| 2225 | Klemskerke 1 | *P.* × *candicans* | Flandre Occidentale (Belgium) |
| Race 3 | | | |
| 2218 | Hautrage-Etat | *P.* × *euramericana* | Hainault (Belgium) |
| Race 4 | | | |
| 2205 | Klemskerke 2 | *P.* × *candicans* | Flandre Occidentale (Belgium) |
| 1923 | Rap | *P. trichocarpa* × *P. deltoides* Rap | Bedfordshire (Britain) |
| 2551 | CF | *P. trichocarpa* × *P. tacamahaca* CF | Surrey (Britain) |
| 2553 | GB-5-15 | *P. tacamahaca* BD | Surrey (Britain) |
| 2697[d] | 177 | *P. trichocarpa* Blom | Wageningen (The Netherlands) |
| Race 5 | | | |
| 2755 | Ra3 | *P.* × *euramericana* Regenerata | Aisne (France) |
| 2750[d] | AS1 | *P.* × *euramericana* Regenerata | Aisne (France) |
| Poorly aggressive strains | | | |
| 2753 | AS4 | *P.* × *euramericana* Regenerata | Aisne (France) |
| 1925 | 88-76-22 | *Populus* sp. | Wageningen (The Netherlands) |
| 2752 | AS3 | *P.* × *euramericana* Regenerata | Aisne (France) |
| 1883 | BII | *P.* × *euramericana* Regenerata | Oise (France) |
| *X. p.* pv. *salicis* | | | |
| 2112 | 102 | *S. dasyclada* | Biesbos (The Netherlands) |

[a] Naturally contaminated host plant from which strains were isolated.
[b] Races were designed in the present study.
[c] French Collection of Phytopathogenic Bacteria (CFBP) number.
[d] Races of strains 2697 and 2750 were only putative because these strains provided inconsistant results between the June and September inoculations (described in text).

and identified by the authors. All strains were pathogenic when inoculated into the very susceptible poplar clone S.6-2, except strains 1883 and 1925, which were avirulent and weakly pathogenic on this clone, respectively. All strains are maintained in lyophilized form in the French Collection of Phytopathogenic Bacteria (CFBP), INRA, Angers. To maintain its aggressiveness, standard strain SPm was inoculated each year on the poplar clone Blanc de Poitou and was reisolated just before resistance assays. Strain 2787, used as standard in this study, was reisolation 18 of strain SPm. Strains were grown from freshly revived cultures on slants of YPGA (5 g of yeast extract, 5 g of peptone, 10 g of glucose, and 15 g of agar per liter of distilled water) at 24 ± 1 C. Virulence of the selected strains of *X. populi* was compared by inoculating stems of poplar clones S.3-31 (*P. trichocarpa*), Boelare and S.6-2 (hybrids of *P. trichocarpa* and *P. deltoides*), 712-7 (*P. tremula* × *P. alba*), and Italica (*P. nigra*), which are known to show a wide range of susceptibility to *X. p. populi* strain SPm (Table 2). One clone of *Salix dasyclada* Wimmer (willow) susceptible to the pathovar *salicis* also was inoculated. Plants were grown in a tree plantation close to Angers, France. Wood cuttings were planted in soil during March 1986, and shoots formed by cuttings were inoculated during their second growth season, during June or September 1987.

**Inoculation and disease measurement.** Shoots were inoculated through bark wounds as previously described (28). Two transversal incisions (about 10 × 2 mm), one at 80 cm and the other at 120 cm above soil level, were made in the shoot bark to increase the chance of inoculation success, because inoculations sometimes fail for unknown reasons. *X. populi* must reach the cambial zone to induce disease, as a result incisions were made deep enough to bare the cambium-sapwood limit. Sufficient inoculum consisting of a 48-h-old slant culture resuspended in sterilized distilled water and optically adjusted to $10^8$–$10^9$ cells per milliliter was introduced immediately to fill the wounds and leave visible drops at both ends of the wounds. Negative controls consisting of poplars inoculated with sterilized distilled water were included. Canker severity of lesions was estimated by measuring both the longitudinal extentions, using canker lengths (L) in centimeters, and the lateral extentions, using the girdling index (GI) of Ridé (25,28), which varies from 0 U of GI or uGI (no symptom) to 5 uGI (stem entirely girdled). Data were recorded after 2 yr during November 1989. In 41 instances, cankers entirely girdled the shoots, and the upper part died-back, preventing measurement of L. In these cases, L values were estimated using the length of the longest canker observed for the same clone–strain–date–inoculation height combination. The data used in analysis of variance (ANOVA) were the average canker size of the two inoculations per tree.

**Experimental design and statistical analyses.** The experimental design was a criss-cross that consisted of seven complete blocks arranged adjacently from north to south. In each block, six clones were planted in rows oriented east to west with two consecutive rows for one clone and 50 cm between rows (whole plots). The 20 strains were inoculated in lines perpendicular to rows with 1.50 m between lines (subplots). The southern row of a given clone was inoculated during June and the northern row during September (sub-subplots). In each sub-subplot, therefore, there were two trees, one per date for each clone–strain–block combination. Clone and strain orders were randomized from block to block. For most of the strains, an additional subplot was randomly inoculated in one block. Data recorded in this eighth pseudo-block were not used in the analyses, except in 22 instances to complete missing values of regular blocks. In 6 other instances, no additional values were available in the pseudo-block, and missing values were estimated by the average of the corresponding clone–strain–date combination.

Susceptibility of poplar clones, aggressiveness of strains, and interactions among clones and strains and among clones and strains and dates were analyzed by ANOVA. A mixed model was used with strain, clone, and date as fixed factors and the block as a random factor. The differences between strains, clones, and dates and the strain–clone, strain–date, clone–date, and strain–clone–date interactions were compared with the strain–block, clone–block, date–block, strain–clone–block, strain–date–block, clone–date–block, and strain–clone–date–block interactions, respectively. The degree of freedom (df) of the mean square of the clone–strain–date–block interaction was reduced to compensate for the estimation of 6 missing values for both GI and L and, additionally, for the L values of 41 estimated values corresponding to totally girdled shoots. On the other hand, because the L values appeared to be distributed like a Poisson's law, a square-root transformation ($\sqrt{L}$) was done to homogenize the variances (4). No transformations were done for the GI values, which were used as they were.

Exploration of a hypothesized race was performed after calculating the various strain–clone interaction values : $I_{sc} = X_{sc} - X_{s.} - X_{.c} + X_{..}$, in which $X_{sc}$ was the average size of the canker formed by *X. populi* strain *s* and the poplar clone *c*, $X_{s.}$ was the average of strain *s*, $X_{.c}$ was the average of clone *c*, $X_{..}$ was the general average, and $I_{sc}$ was the interaction of strain *s* and clone *c*. The various $I_{sc}$ values were calculated on September data instead of the average of June and September data to emphasize the role in the strain–clone interactions of the clone Italica, which was totally resistant during June. A principal component analysis

TABLE 2. Poplar (*Poplus* spp.) and willow (*Salix* sp.) clones used to test host–pathogen interactions with strains of *Xanthomonas populi*

| Clone | Parental origin | Resistance to strain SPm | Reference |
|---|---|---|---|
| Boelare | *P. trichocarpa* Fritzi Pauley × *P. deltoides* S.1-173 (V5 × V9) | Resistant | 29 |
| S.3-31 | *P. trichocarpa* V26 × *P. trichocarpa* V23 | Susceptible | 29 |
| S.6-2 | *P. deltoides* V5 × *P. trichocarpa* V23 | Very susceptible | 28 |
| 712-7 | *P. tremula* 5815 × *P. alba* 5872 | Resistant | 26 |
| Italica | Clone V450 of *P. nigra* cv. Italica | Very resistant | 28 |
| Sd | Natural clone of *S. dasyclada* | Susceptible to *X. p.* pv. *salicis* | 7 |

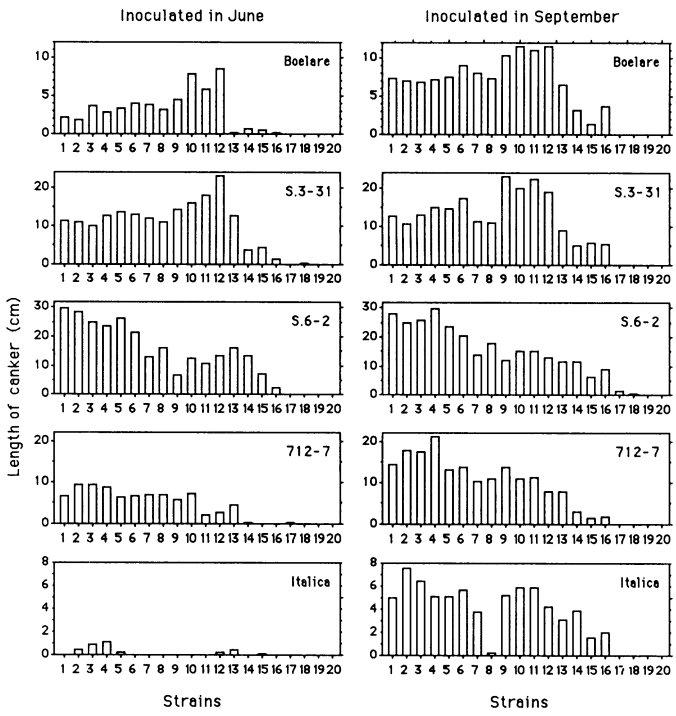


**Fig. 1.** Length of cankers on poplar clones Boelare, S.3-31, S.6-2, 712-7, and Italica inoculated at two different dates with 19 strains of *Xanthomonas populi* pv. *populi* and one strain of *X. p.* pv. *salicis*. Strain numbers correspond to the order in Table 1.

(PCA) was performed on the matrix formed by 10 variables resulting from $I_{sc}$ values calculated for the five poplar clones and for both GI and $\sqrt{L}$ and by 15 *X. populi* strains. Bacterial groups were determined visually from the two-principal-coordinates scattergram of PCA.

The determination of the strain–clone combinations that were the most involved in the clone–strain–date interactions was determined visually from the two-principal-coordinates scattergram of a PCA performed on the matrix of strain–clone–date interaction values. The strain–clone–date interaction value of strain $s$ with clone $c$ at the inoculation date, $d$, was: $J_{scd} = X_{scd} - X_{sc.} - X_{s.d} - X_{.cd} + X_{s..} + X_{.c.} + X_{..d}$, in which $X_{scd}$ was the average canker size formed by strain $s$ on clone $c$ inoculated at date $d$ and the other variables were the according averages. The matrix for PCA consisted of eight variables corresponding to the $J_{sc\ June}$ values of both GI and $\sqrt{L}$ for clones Boelare, S.3-31, S.6-2, and 712-7 and for 15 *X. populi* strains. The two strain–clone combinations that were more distantly related on the first principal coordinate were 2697–Boelare and 2750–S.6-2. Thus, the contrast, $\theta = (Mean_{2697\ Boelare\ June} + Mean_{2697\ S.6\text{-}2\ September} + Mean_{2750\ Boelare\ September} + Mean_{2750\ S.6\text{-}2\ June}) - (Mean_{2697\ Boelare\ September} + Mean_{2697\ S.6\text{-}2\ June} + Mean_{2750\ Boelare\ June} + Mean_{2750\ S.6\text{-}2\ September})$, was calculated and tested for significance in ANOVA. The sum of squares of the residual strain–clone–date interactions was calculated by substracting the sum of squares of $\theta$ from the total strain–clone–date interaction sum of squares.

All statistics were done according to Dagnelie (4). ANOVAs and PCAs were performed using the Stat View SE+Graphics software (Abacus Concepts Inc., Berkeley, CA).

## RESULTS

Strains, clones, and inoculation dates had an effect on canker severity (Fig. 1). Six strains formed very small or no cankers. These strains included avirulent strain 1883, weakly aggressive strain 1925, and strains 2753, 2755, 2750, and 2752, which were previously determined to be more aggressive than those presently found on clone S.6-2. Strain 2697 also was less aggressive than expected but to a lesser extent than were the six former strains. As expected, clones differed widely in their resistance level, and the level of their resistances differed between June and September. For instance, the average canker lengths for Boelare, S.3-31, S.6-2, 712-7, and Italica were 3, 10, 15, 5, and 0.2 cm, respectively, for June inoculations, and 7, 12, 16, 10, and 4 cm, respectively, for September inoculations. On the other hand, there was no canker development on any tested poplar clones inoculated with strain 2112 of *X. p. salicis* (Fig. 1). The willow clone formed cankers with strain 2112 but not with any other strain.

The distributions of canker lengths within each clone (Fig. 1) strongly suggested the occurrence of strain–clone interactions. The bar distributions had nearly the same shape for clones Boelare and S.3-31, although this shape differed for clones S.6-2, 712-7, and Italica. Except for Italica, the distribution shapes seemed similar for June and September inoculations, strongly suggesting no strain–clone–date interactions. These two hypotheses, significant strain–clone interaction and unsignificant strain–clone–date interaction, were tested by ANOVA.

ANOVA requires continuous variables and, at least, approximately normal, with homogeneous variance. However, L and GI values were not continuous close to zero, because they did not show negative values. As a result, the required conditions were not met with several samples, which showed average canker sizes equal or close to zero. This was observed in most cases with strains 1925, 2752, and 1883 and for June inoculations with clone Italica and strain 2753 (Fig. 1). Because the data obtained with some strains were outside the domain of ANOVA application, the analysis was further restricted to clones Boelare, S.3-31, S.6-2, and 712-7 and the 15 remaining strains. The variable distribution was studied, using the data of 72 clone–strain–date combinations that showed no zero values. In these 72 samples, the GI was approximately normal, with no strong correlation between mean and variance, except for five null variances (i.e., same GI for the seven replicates), the extreme variances ($Variance_{GI}max/Variance_{GI}min = 26.8$) indicated that GI variances were homogenous (critical value of Hartley's test: $H_{0.95} = 36.6$ for $P = 72$ and $k = 6$). As a result, the GI values were used as they were used in ANOVA. L was not distributed normally. A significant proportion was observed between variance and mean of L (correlation between variance logarithms and mean logarithms, $r = 0.60$, Fisher's test for the regression factor: $F = 38.3$ for 1 and 71 df, $P < 10^{-4}$), which suggested that L apparently had a Poisson's distribution: $Variance_L = Mean_L$. As suggested by Dagnelie (4) in the case of Poisson's distribution, a square-root transformation can be used to stabilize the variances. By using $\sqrt{L}$ instead of L, the correlation between variance logarithms and mean logarithms was unsignificant ($r = 0.15$, Fisher's test for the regression factor: $F = 1.50$ for 1 and 71 df, $P = 0.22$), and the ratios of extreme variances decreased from $Variance_L max/Variance_L min = 110$ to $Variance_{\sqrt{L}} max/Variance_{\sqrt{L}} min = 83.7$. This indicated a much greater homogeneity of $Variance_{\sqrt{L}}$, even if $Variance_{\sqrt{L}}$ was still heterogenous (critical value of Hartley's test: $H_{0.99} = 72.3$ for $P = 72$ and $k = 6$). However, variance homogeneity is of secondary importance for ANOVA if samples have the same size (4), such as in the present study. As a result, L was transformed by $\sqrt{L}$ for ANOVA.

Three-way ANOVAs for the clones Boelare, S.3-31, S.6-2, and 712-7, the 15 most aggressive strains, and the two inoculation dates indicated significant strain, clone, and date effects and clone–strain interactions for both GI and $\sqrt{L}$ (Tables 3 and 4). A significant clone–strain–date interaction also was found. As suggested by a PCA on strain–clone–date values (not shown), strains 2697 and 2750 seemed to be primarily involved in this second-order interaction. This was confirmed by the contrast $\theta$, which was very significant ($P < 5\ 10^{-4}$), although the residual strain–clone–date interactions were not or were barely significant ($P = 1$ with $\sqrt{L}$; $P = 0.05$ with GI). Thus, except for strains 2697 and 2750, it was assumed that identical strain–clone interactions occurred after June as well as after September inoculations. For this reason, groupings of strains on the basis of a

TABLE 3. Analysis of variance of severity of oozing canker symptoms on poplar clones inoculated with the most aggressive strains of *Xanthomonas populi* pv. *populi*

| Source of variation | df | Sum of squares[a] | Mean squares | F[b] | Probability |
|---|---|---|---|---|---|
| *X. populi* strains | 14 | 308.930 | 22.066 | 38.2 | $<10^{-4}$ |
| Poplar clone | 3 | 534.001 | 178.000 | 132 | $<10^{-4}$ |
| Strain–clone | 42 | 208.184 | 4.957 | 10.71 | $<10^{-4}$ |
| Inoculation date | 1 | 104.603 | 104.603 | 132 | $<10^{-4}$ |
| Strain–date | 14 | 14.399 | 1.029 | 3.31 | $<10^{-4}$ |
| Clone–date | 3 | 64.432 | 21.477 | 23.3 | $<10^{-4}$ |
| Strain–clone–date | 42 | 26.102 | 0.621 | 1.95 | $<10^{-4}$ |
| $\theta$ = (Boelare/S.6-2)-(2697/2750) | 1 | 12.966 | 12.966 | 32.99 | $<10^{-4}$ |
| Residual | 41 | 13.136 | 0.320 | <1 | 1 |
| Block | 6 | 2.192 | 0.365 | | |
| Strain–block | 84 | 48.471 | 0.577 | | |
| Clone–block | 18 | 24.260 | 1.348 | | |
| Strain–clone–block | 252 | 116.655 | 0.463 | | |
| Date–block | 6 | 4.767 | 0.794 | | |
| Strain–date–block | 84 | 26.117 | 0.311 | | |
| Clone–date–block | 18 | 16.574 | 0.921 | | |
| Strain–clone–date–block | 204[c] | 80.245 | 0.393 | | |

[a] Calculated from the square root of canker length on clones Boelare, S.3-31, S.6-2, and 712-7.

[b] Main factors, strains, clones, and dates were fixed; their effects and their various interactions were each compared to their own interaction with the random-factor blocks. For instance, the mean square of the strain–clone interactions was divided by the mean square of the strain–clone–block interactions: 4.957/0.463 = 10.71 for 42 and 252 df.

[c] 204 = 252 − 7 − 41 for seven estimated missing values and 41 estimated canker lengths of entirely girdled shoots.

similar strain–clone interaction to look for bacterial races was done using September results instead of the average of June and September results. This allowed us to include Italica in the race-discrimination analysis.

The PCA principal coordinate, F1, which represented 53% of the matrix variance of clone–strain interaction values, ranked strains according to their differential virulence to Boelare and S.3-31 versus S.6-2 and 712-7. The second coordinate, F2, which represented 22% of the matrix variance, ranked strains according to their differential virulence to Italica versus the four other clones (Fig. 2A). Five groups of strains could be visually discriminated (Fig. 2B). Strains 2181, 2221, 2210, and 2787 (F1 $< -0.5$) were very virulent on S.6-2 and 712-7 and partially avirulent on Boelare and S.3-31. Strains 2220, 2219, and 2225 ($-0.2 <$ F1 $< 0.2$; $-0.2 <$ F2 $< 0.2$) showed no differential virulence with the five tested clones. Strains 2553, 2551, 2205, 2697 and 1923 (F1 $> 0.5$) were very virulent on Boelare and S.3-31 and partially avirulent on S.6-2 and 712-7. Strain 2218 (F2 $< -0.5$) was almost totally avirulent with Italica and showed no differential virulence with the four other clones. Strains 2755 and 2750 (F2 $> 0.5$) were more virulent, relatively, on Italica than on the other tested clones. These groups were considered as races: race 1 was represented by strains 2787, 2181, 2210, and 2221; race 2 by strains 2219, 2220, and 2225; race 3 by strain 2218; race 4 by strains 1923, 2205, 2551, 2553, and 2697; and race 5 by strains 2755 and 2750. Except for strains 2697 and 2750, this classification was valid for June as well as for September inoculations.

The average Ls and GIs were calculated with the five putative races—strains 2750 and 2697 were not taken into account, and averages on Italica were calculated with September data only (Fig. 3). Race 3 (i.e., strain 2218) was totally avirulent on the clone Italica. However, the differences of canker severity provided by the other races on the same clone were generally small in amplitude when compared to the clone average. The races forming the larger and longer cankers were either race 1 or 4 depending on the tested clone. Considering the shorter cankers as the phenotype of an incompatible reaction, Table 5 shows that clones resistant to each main race occurred. The differences between races 1 and 4 in compatible and incompatible reactions were about $\pm 0.8$ uGI and $\pm 0.8$–$1.8$ $cm^{0.5}$.

TABLE 4. Analysis of variance of severity of oozing canker symptoms on poplar clones inoculated with the most aggressive strains of *Xanthomonas populi* pv. *populi*

| Source of variation | df | Sum of squares[a] | Mean squares | F[b] | Probability |
|---|---|---|---|---|---|
| *X. populi* strains | 14 | 173.086 | 12.364 | 30.8 | $<10^{-4}$ |
| Poplar clone | 3 | 934.122 | 311.374 | 335 | $<10^{-4}$ |
| Strain–clone | 42 | 89.746 | 2.137 | 6.56 | $<10^{-4}$ |
| Inoculation date | 1 | 67.499 | 67.499 | 333 | $<10^{-4}$ |
| Strain–date | 14 | 8.862 | 0.633 | 1.96 | 0.025 |
| Clone–date | 3 | 68.175 | 22.275 | 57.5 | $<10^{-4}$ |
| Strain–clone–date | 42 | 17.131 | 0.408 | 1.67 | 0.01 |
| $\theta$ = (Boelare/S.6-2)-(2697/2750) | 1 | 3.047 | 3.047 | 12.5 | $5\ 10^{-4}$ |
| Residual | 41 | 14.084 | 0.344 | 1.41 | 0.05 |
| Block | 6 | 4.699 | 0.783 | | |
| Strain–block | 84 | 34.474 | 0.410 | | |
| Clone–block | 18 | 16.718 | 0.929 | | |
| Strain–clone–block | 252 | 82.041 | 0.326 | | |
| Date–block | 6 | 1.216 | 0.203 | | |
| Strain–date–block | 84 | 27.701 | 0.323 | | |
| Clone–date–block | 18 | 7.104 | 0.395 | | |
| Strain–clone–date–block | 245[c] | 59.676 | 0.244 | | |

[a] Calculated from the girdling index on clones Boelare, S.3-31, S.6-2, and 712-7.

[b] Main factors, strains, clones, and dates were fixed; their effects and their interactions were each compared to their own interaction with the random-factor blocks. For instance, the mean square of the strain–clone interactions was divided by the mean square of the strain–clone–block interactions: 2.137/0.326 = 6.56 for 45 and 252 df.

[c] 245 = 252 − 7 for seven estimated missing values.

## DISCUSSION

In this study, a significant and consistent differential interaction has been noted between *Populus* clones and strains of *X. p. populi*. The interaction was quantitative, and the significance of the results was assessed by ANOVA, using estimations of the canker extension on infected shoots in both width (by GI) and length (by L). A square-root transformation of L ($\sqrt{L}$) was done to stabilize and homogenize the variance. There was no biological consideration in measuring the expression of L in centimeters rather than in square roots of centimeters; such a transformation is familiar in statistics when variables seem to be distributed as a Poisson's law (4). Mather (19) indicated there is no reason why we should not transform the results if by doing so analysis of its results are made more useful or meaningful in some way. We verified, however, that ANOVA performed with $\sqrt{L}$ rather than with L minimized the *F* values of the clone–strain interaction (data not shown), indicating that the transformation did not affect

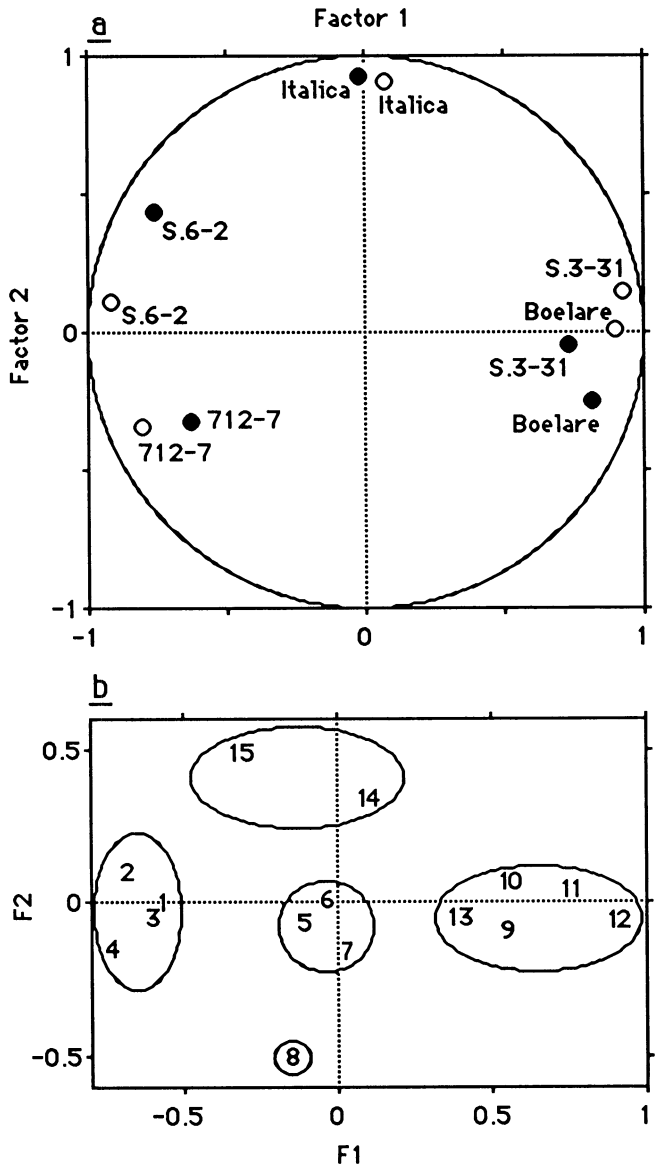


**Fig. 2.** Two principal coordinates (F1 and F2) of the principal component analysis performed on the *Xanthomonas populi*–poplar clone interaction values. **A,** Distribution of the variables on the correlation circle; ○ indicates girdling index, ● indicates canker length. **B,** Distribution of the virulence patterns of the *X. populi* strains on F1 and F2. Numbers correspond to the order in Table 1.

the critical test of the hypothesis in a favorable manner. Very similar patterns were obtained with GI and $\sqrt{L}$ (Fig. 3). Generally, $\sqrt{L}$ seemed to discriminate the race differences better than did GI on some clones (e.g., clone S.3-31 [Fig. 3]), probably because GI admits only discrete values limited between 0 and 5, although L is continuous and not limited for the longest cankers. Nevertheless, GI allows the comparison of our data with previous surveys in which only GI was usually recorded.

The differential virulence of strains was demonstrated in two separate inoculation tests conducted during June and September. During June, poplars are more resistant, whereas during September, at the time of bud closure, poplars are very susceptible (28). Because the difference in the overall severity of disease between June and September did not affect the clone–strain interaction for almost all strains, we concluded that the environment did not influence the interaction. At the same time, the location of the inoculations at 80 or 120 cm above soil level had no significant effect; in particular, there was no correlation between height on the tree and canker length, and height did not influence the clone–strain interaction (data not shown). Specific clone–strain disease reactions demonstrated in this study were similar to canker reactions observed in preliminary surveys (28,29). For instance, strain 1923 was particularly virulent on the very resistant clone Fritzi Pauley (28), and we demonstrated that strain 1923 was very virulent on Boelare, a descendant of Fritzi Pauley. Moreover, the Belgian strains used in this study were chosen after an initial screening on Boelare and Ghoy (29). According to this screening, we confirmed that strain 2205 (but not strain 2225) was more virulent on Boelare. Furthermore, we showed that strains 1923 and 2205 have similar patterns of virulence and probably belong to the same bacterial group. On the other hand, strains 2755 and 2750 exhibited a specific virulence pattern suggesting that these strains belong to a particular race. However, the differential interactions obtained by Ridé and Ridé (27) with strains 2753, 2755, 2750, and 2752 could not be confirmed in the present study, because several of these strains have lost most of their aggressiveness and, therefore, could not be compared by ANOVA.

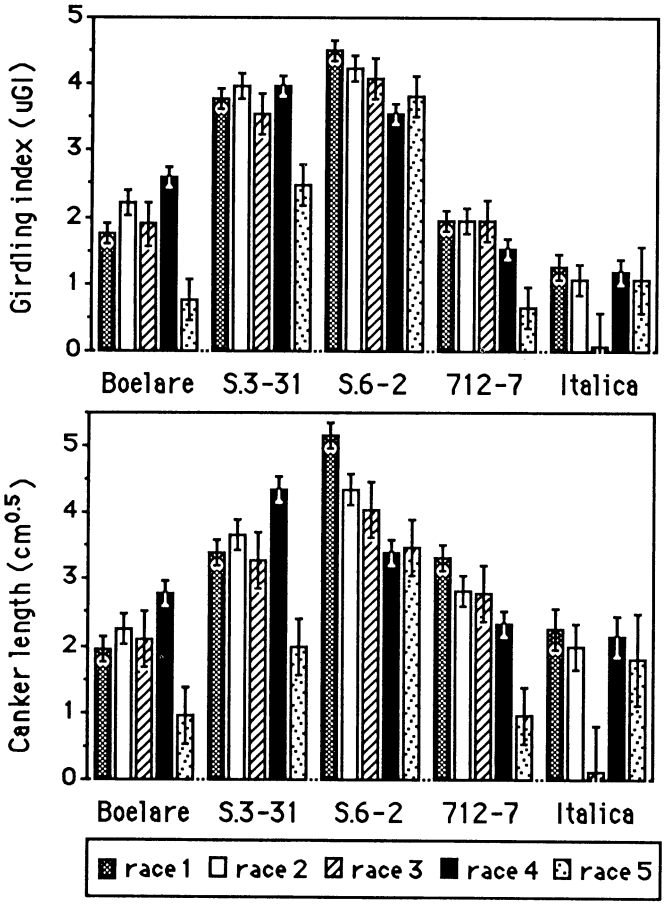


**Fig. 3.** Average canker severity produced by putative races of *Xanthomonas populi* pv. *populi* on poplar clones Boelare, S.3-31, S.6-2, 712-7, and Italica. Boelare, S.3-31, S.6-2, and 712-7 data were averages of June and September inoculation results. Italica data were averages of September inoculations. Bars indicate the 95% confidence interval of the averages.

We do not know if the loss of pathogenicity of *X. p. populi* strains, which has already been described for strain 1883 (27), is the result of a genetic event such as mutation, deletion, plasmid transfer, or selection within a heterogenous population in vitro. The latter hypothesis may be valid because *X. populi* is difficult to isolate, and Danilevicz and Siwecki (5) indicated that the *X. p. populi* colonies did not result from the growth of a single cell but resulted from a cell-cell cooperation phenomenon. As a result, it is possible that some strains were originally composed of a mixture of pathogenic and poorly pathogenic bacteria. In vitro, in absence of a selective pressure for pathogenicity, the genetic drift may have sometimes led to the selection of poorly pathogenic populations. To avoid such a genetic drift, most of the strains used in this study are now maintained on their original host at the Poplar Research Center of Grammont, Belgium. Nevertheless, the majority of the strains have retained their aggressiveness after repeated subculturing.

Host–pathogen interactions suggest the occurrence of physiologic races characterized by an incompatible relationship (resistant–avirulent) on a specific host genotype (10). A high level of resistance was exhibited by Italica when inoculated with strain 2218. However, most of the incompatible relationships found in the present study were characterized by reduced amount of disease rather than by total avirulence. Therefore, bacterial groups were established by visual inspection of PCA on strain–clone interaction values. There is no rigorous method to test the validity of these groupings, and analyses beyond the ANOVA *F* test of the principal analysis are only exploratory. Thus, the present racial classification is strictly putative. Nevertheless, the present strain groupings in the designated races 1 and 4 seem to be consistent with putative classifications deduced from other experiments (28,29). According to what has been proposed by Mew (20) for *X. c. oryzae* on rice, we propose to designate as races the bacterial groups of *X. populi* that gave partial avirulence with differential poplar hosts. We remarked that, according to De Kam's definition (6,7), these races typically belonged to pathovar populi because they formed cankers with poplars and not willow.

A pathogenic race is a taxon of parasites characterized by specialization to different cultivars of one host species (16). Differential interactions are more likely controlled by monogenic or oligogenic rather than polygenic traits in both the pathogen and the host plant in most pathosystems (33). Thus, it is possible that the differences between races of *X. populi* are oligogenic. In such a case, Table 5 suggests the occurrence of at least two gene-for-gene systems: one controlling resistance to race 1 and the second controlling resistance to race 4. In some pathosystems, however, races are specialized to different host species rather than to cultivars belonging to the same species with a genetic determinism, which appears to be more complex (16,22). The poplar clones used in the present study were derived from different species of *Populus*. The clones susceptible to race 1 and resistant to race 4 were: 712-7, which belongs to the *Leuce* section that is sexually incompatible with the *Aigeros* or *Tacamahaca* sections; and S.6-2 and Ghoy, which both originate from the same *P. deltoides* mother, V5 (the resistance pattern of Ghoy was determined in a previous study [29]). Conversely, the clones resistant to race

TABLE 5. Patterns of poplar clone–*Xanthomonas populi* race interactions of the two most virulent races

| | Clone | | | | |
|---|---|---|---|---|---|
| Race | Boelare | S.3-31 | S.6-2 | 712-7 | Italica |
| 1 | –[a] | – | + | + | – |
| 4 | + | + | – | – | – |

[a] –, incompatible reaction (resistant host, avirulent pathogen); +, compatible reaction (susceptible host, virulent pathogen).

1 and susceptible to race 4, Boelare and S.3-31, originated from *P. trichocarpa* mothers, Fritzi Pauley and V26, respectively. In addition, unpublished results strongly suggest that other pure *P. trichocarpa* clones like Fritzi Pauley and CF or clones having *P. trichocarpa* as the mother, such as Beaupré, have the same resistance pattern as Boelare and S.3-31. This suggests that susceptibility to race 4 could be specific to *P. trichocarpa*. If this is true, the resistant clones found among American provenances of *P. trichocarpa* (18) could be particularly susceptible to race 4, because resistances were regularly assessed by using only the standard strain SPm, which belongs to race 1 (2787 is a reisolation of SPm). Thus, *P. trichocarpa* clones should be tested according to priority for their resistance to race 4. We noticed, however, that clone S.6-2 has several ancestors in common with clones Boelare and S.3-31 (i.e., *P. deltoides* V5 and *P. trichocarpa* V23, respectively [Table 2]), even though S.6-2 and Boelare (or S.3-31) are dissimilar in patterns of resistance. This suggests that resistance to race 1 is amenable to the *P. trichocarpa* genome through hybridizations with *P. deltoides*.

Races 1, 2, 3, and 5 were found in continental Europe, and race 4 was isolated in Britain in a very limited area located close to the Belgian coast. Strain SPm (represented by 2787 in this study), the standard strain for routine disease assessments in France, belongs to race 1. The strains used in Belgium, the Netherlands, and Germany for the same purposes also belong to race 1 because the poplar-resistance assessments performed in these countries were always comparable to those performed in France (8,12,28–30). Probably all poplar clones selected in continental Europe for their resistance to *X. p. populi* were assessed with race 1 strains. However, the resistance may remain stable in continental Europe for three reasons. First, up to now, the alone aggressive strain of race 4 originating from the continent was strain 2205, which was isolated in a dune strand that is not a poplar-plantation district. Second, the differences in canker severity between races were generally small in amplitude. This could change the assignation in resistant classes of clones found to be only moderately resistant, however. Actually, an average of 2 uGI was usually retained as the discriminant threshold between susceptible and resistant clones. Thus, a susceptible clone like S.3-31 would be eliminated even if tested with a race 1 strain. Third, poplars are screened for resistance during September, a physiological period of high susceptibility, although natural infections occur during May–June. So, if race 1-resistant poplars were infected by race 4 strains, for instance in Britain, cankers would be more severe than those previously observed but limited as shown with Boelare in this study or with Fritzi Pauley (28). Because limited cankers are known to rapidly heal and close up (X. Nesme, S. Ridé, and M. Ridé, *unpublished data*), the breakdown of race 1 resistance would probably have only limited consequence on poplar-wood quality. However, we strongly suggest the assessment of the resistance level to race 4 of all new poplar clones prior to their commercial planting in Britain, especially for *P. trichocarpa* clones, which were only moderately resistant to race 1 strains.

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