

Wettability of Poplar Leaves Influences Dew Formation and Infection by *Melampsora larici-populina*

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ABSTRACT

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The wettability of leaves of 60 poplar (*Populus* spp.) clones from sections *Aigeiros* (black) and *Tacamahaca* (balsam) and their hybrids was assessed by measuring the contact angle of calibrated water droplets on their abaxial leaf surface. The frequency and duration of dew on these clones were recorded in two nurseries. Black poplar leaves had the highest wettability, with relatively flat-shaped water droplets and a short drying time, in both the laboratory and nursery. Conversely, round-shaped water droplets on balsam poplars dried more slowly in the laboratory. In the nursery, dew was more frequent and persisted longer. First-generation intersectional hybrids behaved like balsam poplars, but successive backcrosses with black poplar (*P. deltoides*) resulted in clones with leaves that were more wettable and possessed less frequent and persistent dew. Infection by *Melampsora larici-populina* on two hybrid clones ('Robusta' and 'Beaupré'), assessed by the number of uredinia in the laboratory, was dependent on the duration of leaf moisture. Maximum infection occurred when water was present on the leaves for 12 h. Leaf wettability should be considered as an additional trait when breeding poplar for durable resistance to *M. larici-populina*, as this characteristic is likely to be a useful defense against all pathotypes of the pathogen.

Additional keywords: poplar rust

Hybrid poplars are fast growing trees, selected for their vigor (heterosis) and their wood quality, especially for veneer and plywood. Trees are cultivated as monoclonal stands and may therefore be susceptible to high disease levels. In practice, a small number of cultivars are widely used, and two-thirds of the stands in France consist of only two or three cultivars. This homogeneity contrasts with the natural diversity in the genus *Populus* including many interspecific crosses. The genus *Populus* is divided into six sections (4). In Europe, most of the cultivated poplars belong to two hybrid species: *P. × euramericana* (Dode) Guinier (syn. *P. × canadensis* Moench) and *P. × interamericana* Brockh. (syn. *P. × generosa* Henry). The first resulted from the hybridization of the American cottonwood (*P. deltoides* Marsh.) with the European black poplar (*P. nigra* L.). The second is mainly a man-made hybrid between *P. deltoides* and the American balsam poplar (*P. trichocarpa* Torr. & Gray). *P. nigra* and *P. deltoides* belong to the section *Aigeiros* (later called

black poplars), while *P. trichocarpa* is a member of the section *Tacamahaca* (later called balsam poplars). Balsam poplars also include *P. balsamifera* L. (from North America) and *P. maximowiczii* A. Henry (from Asia). This large diversity among poplars has allowed selection of new genotypes after major disease epidemics (i.e., bacterial canker, rust, or *Marssonina brunnea*).

Poplar rust, caused predominantly in Europe by *Melampsora larici-populina* Kleb., is a major concern after the breakdown of total resistance of widely planted cultivars (13,18). Most of the cultivars were selected for this type of resistance to this *Melampsora* (13). On the widely planted cultivar *P. × interamericana* 'Beaupré', growth restriction due to rust was estimated to be 20 to 30% of the biomass in the first year of infection and up to 50 to 60% in the following years (6). Many young rust-infected trees were killed after subsequent infection by *Discosporium populeum* Sacc. Infection by *M. larici-populina* in the field, which occurs on the abaxial leaf surface, was more severe on *P. × interamericana* than on *P. × euramericana* cultivars, whereas components of partial resistance (latent period and infection) in laboratory tests (under continuous leaf wetness) were similar for 'Beaupré' and *P. × euramericana* 'Robusta' (19). To protect mature stands until harvest, spraying fungicide became necessary, mainly on

P. × interamericana cultivars. New plantations are currently established with previously selected cultivars (mainly *P. × euramericana*), which are less vigorous but are reasonably resistant in the field.

Rusts are favored by dew and leaf wetness. In agriculture, the relationship between dew duration and infection is well documented. For *Puccinia chondrillina* on rush skeleton weed, Emge et al. (5) established that the optimal dew period was 16 h at 10 to 21°C. For *Phakospora pachyrhizi*, no rust developed on soybean when dew periods were less than 6 h, and the optimum was 13 h (15). *Gladiolus* infection by *Uromyces transversalis* was severe when leaves were wet for 12 h at 10 to 20°C but slight for 9 h of wetting (1). Maximum severity of *Puccinia xanthii* on *Xanthium occidentale* was observed on plants exposed to a dew period of 24 h. Fewer symptoms developed as a result of shorter (3, 6, or 12 h) or longer (36 h) dew periods (16). On alfalfa, infection efficiency of *Uromyces striatus* increased linearly as the duration of leaf wetness was increased from 4 to 24 h (23). Politowski and Browning (20) determined that the formation of infection structures of *Puccinia coronata* var. *avenae* needed at least 5 h of dew with an optimum at 16 h. Infection by *Uromyces viciae-fabae* on broad beans began with 4 h of leaf wetness but increased with longer periods (10). On poplar, Krzan (11) established a link in the field between average humidity and rust severity, but no temperature effect. Hamelin et al. (7) concluded that rain deficit and high maximum temperatures were not limiting factors for leaf rust epidemics by *Melampsora medusae* f. sp. *deltoidae* on *P. deltoides* when night conditions offered optimal temperature and leaf wetness for infection. The important role of dew in relation to infection was used to develop predictive models for soybean rust (25), wheat stripe rust and leaf rust (3), and rust on perennial ryegrass seed crops (17). Such models also included other parameters such as temperature or light.

Huber and Gillespie (9) published a review about modeling leaf wetness in relation to plant disease epidemiology. They indicated that dew duration was a complex phenomenon that involved several factors, such as climate, leaf wetness, and plantation structure. At least for rusts, few investigators have given attention to leaf wettability in relation to infection. According

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to Cook (2), peanut leaves were less susceptible to rust when they were less wettable. On artificial substrates, adhesion of urediniospores and urediniospore germ-lings of *Uromyces appendiculatus* depended on surface wettability and was more efficient when wettability was less than 30% (22). The degree of germling contact was closely correlated with hydrophobicity of the leaf surface.

Leaf wettability is generally measured using the contact angle between water droplets and leaf surface. Holloway (8) reviewed this topic for many plants, but it did not include the Salicaceae (*Salix* or *Populus*). Of the species reviewed, *Rumex obtusifolius* was the most wettable (39° to 40°), while the maximum contact angle was reached by *Eucalyptus* (167° to 170°). Martin and Juniper (14) proposed a link between leaf wettability and dew aspect. On a wettable leaf surface, below the ambient temperature in a saturated atmosphere, a continuous film of water was formed. Under the same conditions, an unwettable surface was covered with small droplets of water.

Dew formation and persistence in relation to leaf wettability (and therefore contact angle) are presumably involved in rust infection, but very few researchers have attempted to establish a relationship between these characteristics. On *Pinus monticola* seedlings, Woo et al. (24) examined 14 needle traits including epistomal wax degradation, weight of wax per dry weight of needle, contact angle of water droplets, and the morphology of wax. They suggested that wax aspect and amount had an effect on surface wettability and suggested that these characteristics should be considered when breeding for resistance to *Cronartium ribicola*.

In our work, leaf wettability, dew formation and its persistence, and correlation with *M. larici-populina* infection on poplar were explored using a multifactor approach and taking poplar variability into account (pure species, hybrids, and their backcrosses).

MATERIALS AND METHODS

Plant materials. Cuttings of poplar clones were grown in containers in the glasshouse and the nursery in Nancy (eastern France) and in Guémené-Penfao (western France) as monoclonal plots of at least eight plants per clone. The clones tested are listed in Table 1 and include three pure species, *P. deltoides* (D), *P. nigra* (N), and *P. trichocarpa* (T), and seven hybrid combinations, *P. deltoides* × *P. nigra* (D × N, syn. *P. × euramericana*), *P. balsamifera* × *P. deltoides* (B × D, syn. *P. × jackii* Sargent), *P. trichocarpa* × *P. maximowiczii* (T × M), *P. trichocarpa* × *P. deltoides* (T × D or D × T, syn. *P. × interamericana*). Also, backcrosses of the latter with *P. deltoides* or *P. trichocarpa* were studied.

Many hybrids resulted from controlled crosses carried out by the Instituut voor Bosbouw en Wildebeheer (IBW) in Geraardsbergen (Belgium), and some by AFOCEL (Charrey-sur-Saône, France) and INRA (Orléans, France). Cultivars included in this study were all selected for a reasonable level of partial resistance to *M. larici-populina*. Exceptions included 'Robusta', a relatively old cultivar, and 'Beaupré', a cultivar that has lost its complete resistance after 15 years of cultivation and now exhibits a low level of partial resistance. None of the other clones (i.e., unregistered) were selected for partial resistance. No clone or cultivar had been

examined previously for leaf wetness. This distinction between criteria (selected or not) was taken into account when the comparison between parameters was made. The leaves were numbered according to the leaf plastochron index (LPI) (12), leaf number one being the youngest fully expanded leaf.

Contact angle. In the laboratory, droplets of 2 µl of deionized water were deposited onto the abaxial (underside) face of small pieces of rust-free leaves (five leaves with LPI between 5 and 15), which had been collected in the nursery in June. Areas with veins were avoided. Contact angle with the leaf surface was measured with an inverse stereo binocular equipped with a goniometer. At least 20 droplets per clone were measured, except for clones in Figure 1 (100 droplets per clone).

Drying time in the laboratory. Calibrated (4 µl) droplets of deionized water were deposited on the abaxial surface of leaf disks (30 mm diameter) cut from leaves of poplars that had been cultivated in the nursery. Leaf disks were subjected to a laminar air flow at 28°C (air speed approximately 0.4 m/s). Each disk received 4 droplets and each clone was represented by 5 leaf disks. The time necessary for drying was measured by visual observation of the droplet evaporation. Clones from seven different species or hybrid combinations were compared: 'Italica' (N), 'Robusta' (D × N), 'Beaupré' (T × D), 'Fritzi Pauley' (T), 11 (T × D) × D clones (B 76022-4, B 76025-4, B 81015-136, B 81018-208, B 81025-442, B 82003-924, B 82008-3453, B 82009-1403, B 82011-1686, B 82014-1686, B 82022-1984, B 82023-2053.), 4 (T × D) × T clones, and 5 T × M clones.

Dew presence and persistence on poplar leaves in the nursery. All the observa-

Table 1. Poplar clones included in the study

Species	Species code ^a	Section	Clone ^b
<i>Populus deltoides</i>	D	<i>Aigeiros</i>	87B12, 'A79', 'Alcinde', 'Peoria'
<i>P. nigra</i>	N	<i>Aigeiros</i>	'Ankum', 'Italica'
<i>P. deltoides</i> × <i>P. nigra</i> (= <i>P. × euramericana</i>)	D × N	<i>Aigeiros</i>	'Brabantica', 'Ghoy', 'I-214', 'Luisa Avanzo', 'Ogy', 'Robusta'
<i>P. trichocarpa</i>	T	<i>Tacamahaca</i>	'Fritzi Pauley', 'Trichobel'
<i>P. trichocarpa</i> × <i>P. maximowiczii</i>	T × M	<i>Tacamahaca</i>	B 75023-18, B 75023-21, B 75023-23, B 75023-31, B 75023-41
<i>P. balsamifera</i> × <i>P. deltoides</i> (= <i>P. × jackii</i>)	B × D	Intersectional	'Aurora'
<i>P. trichocarpa</i> × <i>P. deltoides</i> (= <i>P. × interamericana</i>)	T × D	Intersectional	27B09, 'Beaupré', 'Boelare', 'Hazendans', 'Hoogvorst', 'Raspalje'
<i>P. deltoides</i> × <i>P. trichocarpa</i> (= <i>P. × interamericana</i>)	D × T	Intersectional	'Donk'
<i>P. × interamericana</i> × <i>P. trichocarpa</i>	(T × D) × T	Intersectional	AFO 86 A' 34, AFO 86 A' 35, AFO 86 A' 36, AFO 86 G 09
<i>P. × interamericana</i> × <i>P. deltoides</i>	(T × D) × D	Intersectional	B 76022-4*, B 76025-4*, B 76028-5*, B 76029-15*, B 76032-6, B 81014-102*, B 81015-136*, B 81017-197*, B 81018-208*, B 81024-394*, B 81025-442*, B 82010-1509, B 82010-3494, B 82003-924, B 82005-3494, B 82008-3453, B 82009-1403, B 82011-1686, B 82012-3508, B 82014-3522, B 82022-1984, B 82023-2053, B 82025-2178, B 82027-3620
(<i>P. × interamericana</i> × <i>P. deltoides</i>) × <i>P. deltoides</i>	((T × D) × D) × D	Intersectional	B 01052/1, B 01056/28, B 01059/2, B 01061/4, B 01062/81

^a Code for pure species: D = *P. deltoides*, N = *P. nigra*, T = *P. trichocarpa*, M = *P. maximowiczii*, B = *P. balsamifera*.

^b Origin of clones: AFO = AFOCEL; B = IBW (Belgium); 87B12 and 27B09 = INRA Orléans. * = 'Beaupré' as parent. Cultivars: names between quotation marks.

tions were carried out on 1 year shoots, generally on leaves with LPI 7. In 2000 at the Guémené-Penfao location, several clones were visually assessed four times per day (9 A.M., 11 A.M., 2 P.M., and 4 P.M.) for the presence of dew on their leaves (abaxial surface). In Nancy during the summer of 2000, 20 clones were observed for dew presence at 9 A.M., 11 A.M., 1 P.M., and 3 P.M. in the nursery between 26 June and 7 September. For two clones ('Hazendans' and 'Hoogvorst'), leaves were observed on plants cultivated in different nursery beds to take a possible microclimate effect into account. During this period, dew occurred on 38 days of the 52 observation days. Dew frequency was defined as the number of days with dew present on a clone at 9 A.M. related to the total number of days in the observation period. During the summer of 2001, emphasis was placed on (T × D) × D hybrids.

Effect of leaf moisture duration on infection by *M. larici-populina*. Two cultivars, *P. × euramericana* 'Robusta' and *P. × interamericana* 'Beaupré', were grown in the glasshouse (two plants per clone) and 30-mm disks were cut from their leaves (LPI 7 to 15) and inoculated in the laboratory on the abaxial surface. Inoculation was carried out by spraying a urediniospore suspension (6,500 spores per ml) of *M. larici-populina* isolate 98AG31 (pathotype 3-4-7), which is pathogenic on both clones. Disks were incubated at 20°C,

floating on water in six-well cell culture plates under continuous fluorescent light (550 lux). Disks were removed from plates after each incubation period (from 2 to 24 h), dried under a laminar air flow at 20°C, and then replaced in plates. Eight disks from 'Beaupré' and six from 'Robusta' were used for each incubation period. The infection by *M. larici-populina* was assessed 10 days after inoculation by counting the resulting uredinia.

Rust infection in the nursery. Leaf infection by *M. larici-populina* was assessed under a natural inoculum on 1 year shoots in the nursery in Nancy. *M. larici-populina* inoculum present in this nursery exhibited a very high pathotype diversity because of the presence of many different poplar clones and the presence of larches (18). A minimum of 50 leaves per shoot and six to eight shoots per clone were scored. Rust scoring was performed using a scale proposed by M. Villar (*personal communication*): from 1 (no visible uredinia on any leaf of the whole shoot) to 9 (uredinia cover more than three-quarters of the surface area of the most heavily infected leaf and more than 25% of the leaves of the whole shoot exhibit this level of infection). Infection was assessed on 'Beaupré', 11 (T × D) × D clones (B 76022-4, B 76025-4, B 81015-136, B 81018-208, B 81025-442, B 82003-924, B 82008-3453, B 82009-1403, B 82011-1686, B 82022-1984, and B 82023-2053) and the four (T × D) × T

clones. None of these clones had been selected previously for partial resistance.

Modifying leaf wettability. An experimental approach was used to test the effect of a wetting agent on infection by *M. larici-populina*. Water droplet contact angles were measured after spraying the abaxial surface of the leaves with several wetting agents: Tween 20 (Polyethylene glycol sorbitan monolaurate, 0.005 and 0.01%), Spreader (Pine resin, 0.025 and 0.05%), Seppic (petrol white oil, 0.05 and 0.1%), gelatin (1 and 2 g/liter), Citowett (alkaryl polyglycol, 0.05 and 0.1%), and Fundation (alkylpolysaccharides, 0.1%). The effect of the wetting agents on urediniospore germination and infection also was tested.

Statistical analyses. For contact angle, drying time, and infection by *M. larici-populina*, data were analyzed using ANOVA with Statgraphics 5.1 software. A linear model was used to relate variables. Frequencies were compared with the G-test or 2I test (Log-likelihood ratio test) as suggested by Sokal and Rohlf (21). For multiple comparisons, a confidence level of 95% was chosen.

RESULTS

Contact angle. The water droplet contact angles varied widely ranging from 42° to 97°. Higher values of contact angle corresponded to round-shaped droplets, i.e., to more hydrophobic leaf surfaces. A

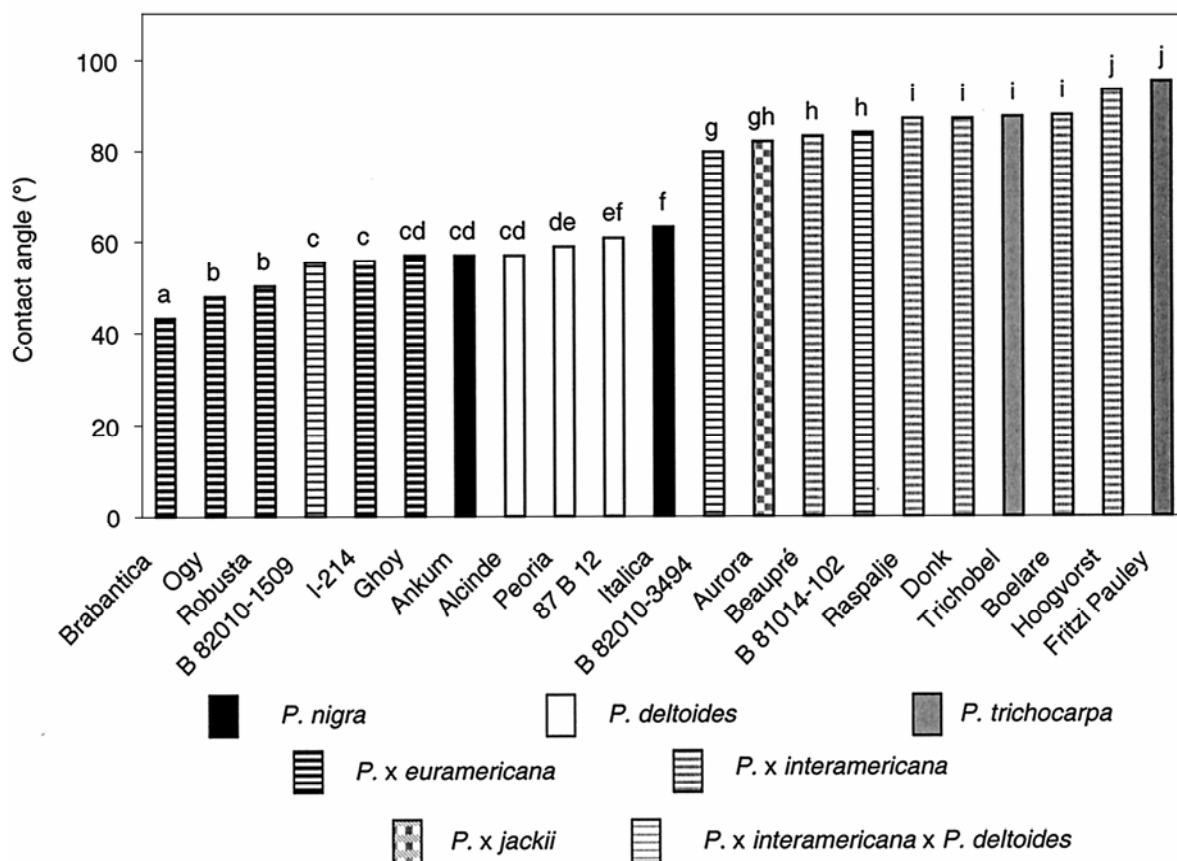


Fig. 1. Comparison of poplar clones for contact angle of calibrated water droplets. Values with the same letter do not differ significantly ($P < 0.05$).

preliminary comparison was carried out for clones from the major species (Fig. 1). The differences among poplar species were important and significant ($F = 612$, 6 df, $P < 0.0001$). Black poplars exhibited the lowest angle values with no significant difference between *P. nigra* (60°) and *P. deltoides* (59°). The mean value (51°) for their hybrids (*P. × euramericana*) was significantly the lowest. Significantly higher values were recorded for pure balsam poplars (*P. trichocarpa*) or their hybrids with *P. deltoides*. All species or hybrids involving a balsam poplar (*P. trichocarpa* or *P. balsamifera*) differed significantly ($P < 0.001$) between themselves and were classified by increasing values as follows: (T × D) × D clones (73°), B × D clone (82°), T × D clones (88°), and T clones (91°). Clone effect was significant ($F = 262.5$, 20 df, $P < 0.0001$) and reflected that of the poplar species (Fig. 1). Within each F1 interspecific hybrid (D × N and T × D), some significant differences between clones were found, but these differences were small. In the backcrosses, more variation was found between (T × D) × D clones (Fig. 1): B 82010-1509 had values in the middle of the range found for D × N (hybrid black poplar) while B 82010-3494 and B 81014-102 had higher values, typical of those of the balsam poplars.

As a result of this initial evidence of variability between (T × D) × D hybrids, more clones from this combination were studied in comparison with 'Beaupré' (T × D) and some (T × D) × T hybrids. Species

effect was highly significant ($F = 34.23$, 6 df, $P < 0.0001$). Variability was found among (T × D) × D: the angle values ranged from 58° to 90° (Fig. 2) with significant differences among clones. Here again, backcrossing *P. × interamericana* with *P. deltoides* gave rise to clones with reduced contact angles. Conversely, four clones resulting from backcrossing with *P. trichocarpa* ((T × D) × T) provided values of the contact angles between 89° and 95°, which were not significantly different from those of pure *P. trichocarpa* (88°) or of T × D 'Beaupré' (91°). The hybrid between the American and the Asian balsam poplars T × M provided significant variability ($P < 0.05$) among the five clones studied with a range between 79° (B 75023-18) and 97° (B 75023-23).

Five complex hybrids, derived from *P. × interamericana* backcrossed twice with *P. deltoides* ((T × D) × D) × D, were compared with *P. × euramericana* 'Robusta' and *P. × interamericana* 'Beaupré'. Clone effect was significant ($F = 50.1$, 6 df, $P < 0.0001$). All exhibited angle values that were significantly lower than 'Beaupré'. One clone (B 01059/2) gave a significantly different value (67°) that was intermediate between that of 'Beaupré' (86°) and 'Robusta' (63°). Three clones (with angle values of 60°, 62°, and 64°, respectively) were indistinct from 'Robusta', while one clone (B 01052/1) provided angles with significantly lower values (52°) than 'Robusta'.

The effect of leaf position also was significant on clones listed in Figure 1 when

the whole range of LPI was explored ($F = 27.98$, 5 df, $P < 0.0001$). The lowest values were associated with both the youngest and the more mature leaves. Fully expanded leaves (LPI 7) were the most susceptible to *M. larici-populina* and also exhibited the highest values of contact angle.

Drying time of water droplets. A significant species effect was detected ($F = 34.97$, 6 df, $P < 0.0001$; Fig. 3). *P. trichocarpa* 'Fritzi Pauley' needed more than twice the time for droplets to dry (56 min) compared with *P. nigra* 'Italica' (24 min). *P. × euramericana* 'Robusta' (28 min) was indistinct from *P. nigra* and from 11 (T × D) × D clones (a mean of 30 min). All hybrids with a predominant balsam poplar background (four (T × D) × T clones, *P. × interamericana* 'Beaupré', and five T × M clones) formed a homogenous group. Drying time was significantly lower for clones in this group than for *P. trichocarpa* 'Fritzi Pauley' but longer than for clones with black poplar as a major component of their pedigree, including (T × D) × D hybrids.

Dew frequency in the nursery. In Guémené-Penfao, dew frequency was studied on 11 clones between 8 June and 3 August 2000. The analysis revealed a significant clone effect ($G = 22.3$, 10 df, $P = 0.013$). The first group consisted of *P. deltoides* ('Alcinde' and 'A79'), D × N ('Robusta', 'I-214', and 'Luisa Avanzo'), and (T × D) × D clones (B 82010-3494, B 82010-1509, and B 81014-102). Dew was significantly more frequent on clones with a predominant balsam poplar background:

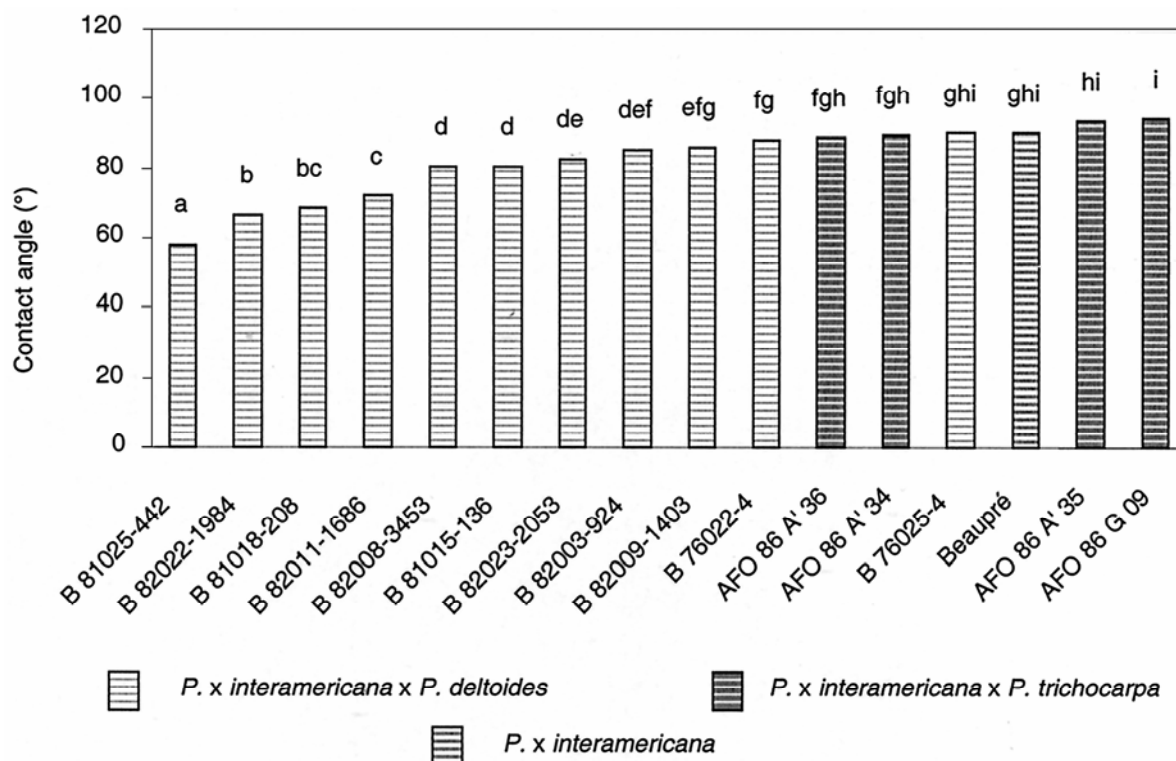


Fig. 2. Variability for contact angle of calibrated water droplets among *P. × interamericana* × *P. deltoides* and *P. × interamericana* × *P. trichocarpa* clones in comparison with 'Beaupré' (*P. × interamericana*). Values with the same letter do not differ significantly ($P < 0.05$).

P. trichocarpa 'Fritzi Pauley', *P. × interamericana* 'Beaupré', and 27B09.

In Nancy (26 June to 7 September 2000), dew was detected on 38 days and a significant clone effect ($G = 239.7$, 21 df, $P < 0.0001$) was noticed (Fig. 4). Dew was significantly more frequent on clones with a strong balsam poplar background (*P. trichocarpa* and *P. × interamericana*) compared with black poplars (*P. deltoides*, *P. nigra* and their hybrids *P. × euramericana*, and $(T \times D) \times D$ hybrids). No significant difference was found for the two cultivars ('Hoogvorst'

and 'Hazendans') represented by plants located in two different beds.

In Nancy in 2001, 22 $(T \times D) \times D$ clones were compared with 'Robusta', 'Fritzi Pauley', and 'Beaupré'. During 42 days of observation within the period 19 June to 18 September, dew was present for 3 days on 'Robusta', 10 days on $(T \times D) \times D$ hybrids, and 23 days on 'Beaupré' and 'Fritzi Pauley'. Clones fell into three significantly different groups ($G = 33.9$, 3 df, $P < 0.0001$), namely 'Robusta', $(T \times D) \times D$ hybrids, 'Beaupré' and 'Fritzi Pauley'.

In Nancy in 2003, the summer was especially warm and dry. Nevertheless, dew was observed on new hybrids between 16 June and 17 July. Dew was significantly less frequent ($G = 68.9$, 6 df, $P < 0.0001$) on a homogenous group of clones ('Italica', 'Robusta', 4 $T \times M$ clones, and 21 $(T \times D) \times D$ clones). The highest dew frequency was on 'Beaupré', while 'Fritzi Pauley' and 4 *P. × interamericana* backcrossed with *P. trichocarpa* were intermediate but not significantly different from 'Beaupré'.

Dew persistence in the nursery. In Guémené-Penfao, between 8 June and 3 August 2000, dew persistence was measured (14 days with dew). There was a significant clone effect ($G = 147.9$, 40 df, $P < 0.0001$) that reflected differences among species. No significant difference was found between *P. deltoides* clones ('Alcinde' and 'A79'), *P. × euramericana* ('Robusta', 'I-214', and 'Luisa Avanzo'), and $(T \times D) \times D$ hybrids, but all these clones differed significantly from clones with a predominant balsam poplar background (longer dew persistence): 'Beaupré' ($T \times D$), 'Fritzi Pauley' (T), and 27B09 ($T \times D$). No significant difference was found between these clones. Results from the most widely planted cultivars are presented in Figure 5.

In Nancy, during June and July 2003, dew was present during 14 days. Dew was detected at 9 A.M. on some $(T \times D) \times D$ hybrids, on all $(T \times D) \times T$ hybrids, and on 'Beaupré' and 'Fritzi-Pauley'. Dew was no longer noticed at 11 A.M. on $(T \times D) \times D$ hybrids but was still present at 3 P.M. on $(T \times D) \times T$ hybrids, 'Beaupré', and 'Fritzi Pauley'.

Effect of duration of leaf moisture on infection by *M. larici-populina*. The duration of a wet period on inoculated poplar

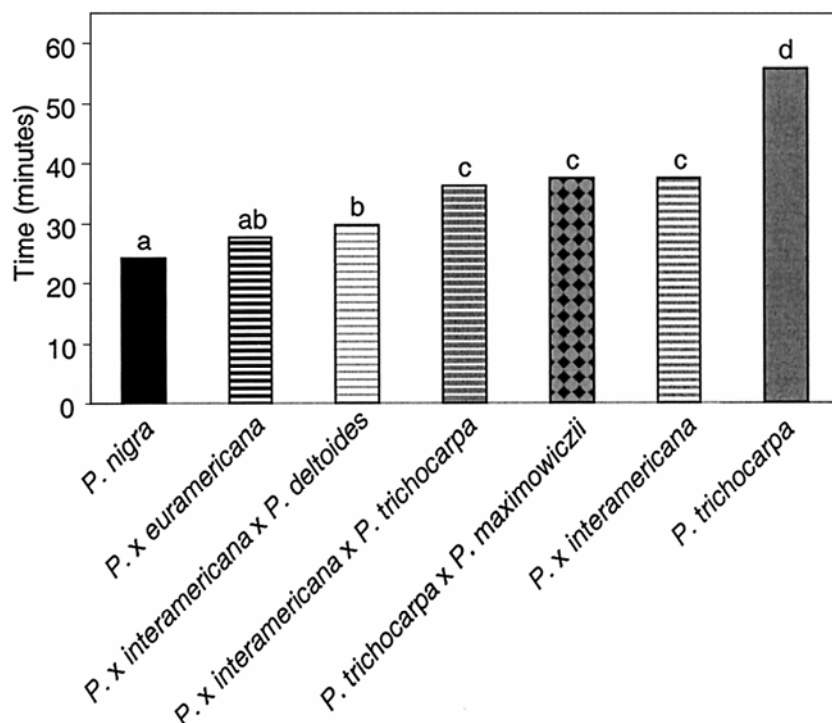


Fig. 3. Time for drying calibrated water droplets on various poplar species and hybrids. Values with the same letter do not differ significantly ($P < 0.05$).

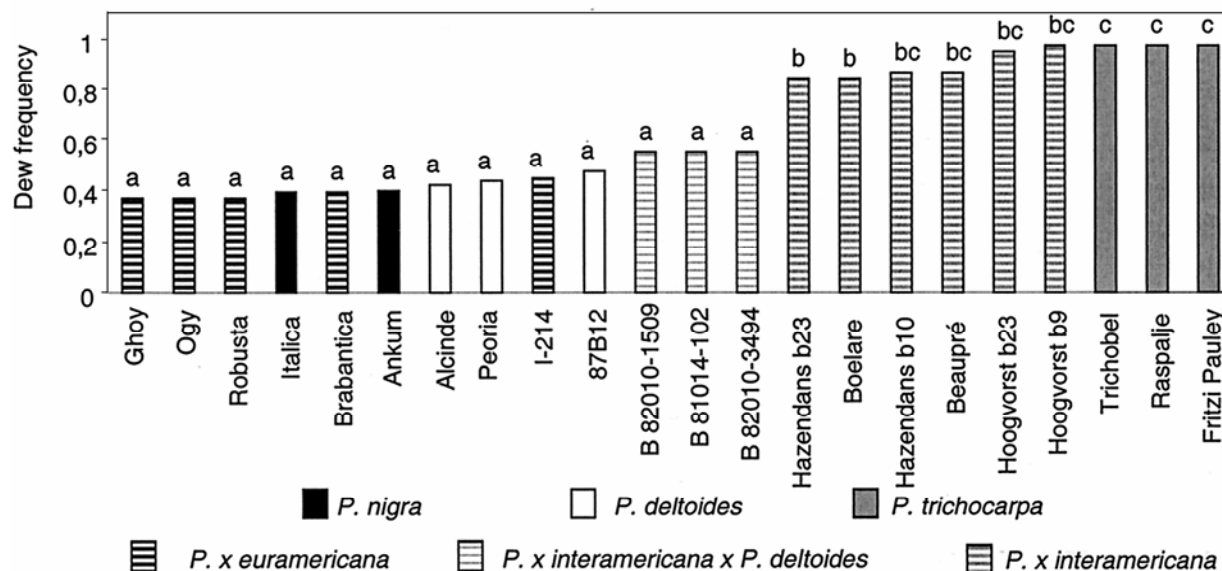


Fig. 4. Relative frequency of dew observed in the nursery in 2000 in Nancy on several poplar clones: b9, b10, and b23 represent different stoolbeds. Values with the same letter do not differ significantly ($P < 0.05$).

leaf disks had a highly significant effect on infection which was assessed by the number of sporulating uredinia ($F = 34.52$, 8 df, $P < 0.0001$). Infection of clones 'Robusta' and 'Beaupré' required a wet period of at least 2 h, reaching a maximum after 12 h (Fig. 6).

Modifying leaf wettability. Tween 20 (0.01%) and Citowett (0.01%) produced the greatest reductions in contact angle values on 'Beaupré' and 'Hazendans', 26 and 22%, respectively. However, these chemicals had a strong depressive effect on infection by *M. larici-populina* as determined by the number of uredinia, reducing infection by 97 and 61% on 'Beaupré' and 'Hazendans', respectively. When tested at higher dilutions, these chemicals had a lower depressive effect on infection, but the modification of contact angle also was small. Gelatin (0.1%) did not affect contact angle, and the other chemicals, used at previously mentioned concentrations, modified angle values only slightly but had a negative effect on rust infection.

Infection in the nursery. At the Nancy site in 2003, infection by *M. larici-populina* was scored 8.3 on 'Beaupré', while $(T \times D) \times D$ clones ranged between 4.8 and 7.3 (mean 5.4) and the $(T \times D) \times T$ clones between 8.2 and 9 (mean 8.6).

Relationships between parameters. Eight clones were common to Nancy and Guémené-Penfao for the assessment of dew presence in 2000: one *P. deltoides* ('Alcinde'), one *P. trichocarpa* ('Fritzi Pauley'), one *P. × interamericana* ('Beaupré'), two *P. × euramericana* ('I-214' and 'Robusta'), and three $(T \times D) \times D$ (B 81014-102, B 82010-1509, and B 82010-3494). Data from these two locations were significantly related ($R^2 = 0.82$).

Frequency of dew was correlated with dew persistence. In Guémené-Penfao, the correlation coefficient between the number of days with dew at 9 A.M. and the number

of days with dew at 11 A.M., 2 P.M., and 4 P.M. were highly significant ($P < 0.01$) for 11 A.M. ($r = 0.91$) and 2 P.M. ($r = 0.91$) and significant ($P < 0.05$) for 4 P.M. ($r = 0.67$). Similar calculations were made for Nancy for the same year, comparing frequency at 9 A.M., 11 A.M., 1 P.M., and 3 P.M. All coefficients were highly significant ($P < 0.01$) with respective r values of 0.96, 0.95, and 0.97.

Dew frequency in Nancy in 2000 was compared with contact angle on the same clones measured in the laboratory. After converting frequency into $\text{Arcsin}\sqrt{\%}$, the best adjustment was:

$$\text{Dew frequency} = 1/(0.041 - 0.0003 \cdot \text{angle}), \text{ with } R^2 = 0.83$$

In 2003, with a dry summer not favorable to dew formation, and on a different

set of poplar clones, the correlation between dew frequency and contact angle was lower but still significant ($P = 0.048$ and $R^2 = 0.17$). The best adjustment was:

$$\text{Dew frequency} = 1/(0.038 - 0.0002 \cdot \text{angle})$$

Under the same conditions, the time for drying droplets in the laboratory was a better predictor of dew frequency on the same set of clones with $P < 0.0001$ and $R^2 = 0.51$. The best adjustment was:

$$\text{Dew frequency} = 16.70 + 0.70 \cdot \text{drying time}$$

A significant relationship was found between contact angle and time for drying ($P = 0.003$, $R^2 = 0.34$).

The relationships between infection by *M. larici-populina* in the nursery and other parameters were studied for *P. × interamericana* 'Beaupré', 11 $(T \times D) \times D$

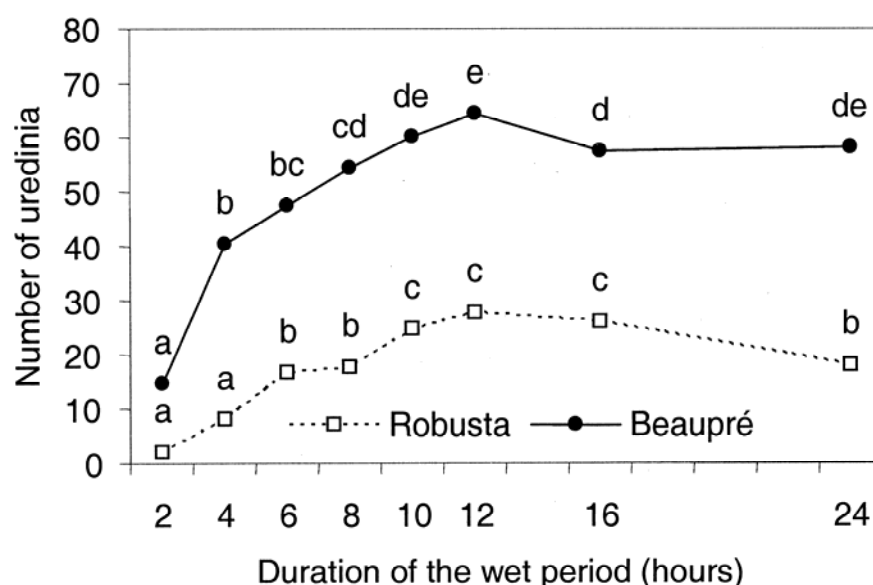


Fig. 6. Effect of the duration of the wet period on infection of two poplar cultivars in the laboratory. Values with the same letter do not differ significantly ($P < 0.05$).

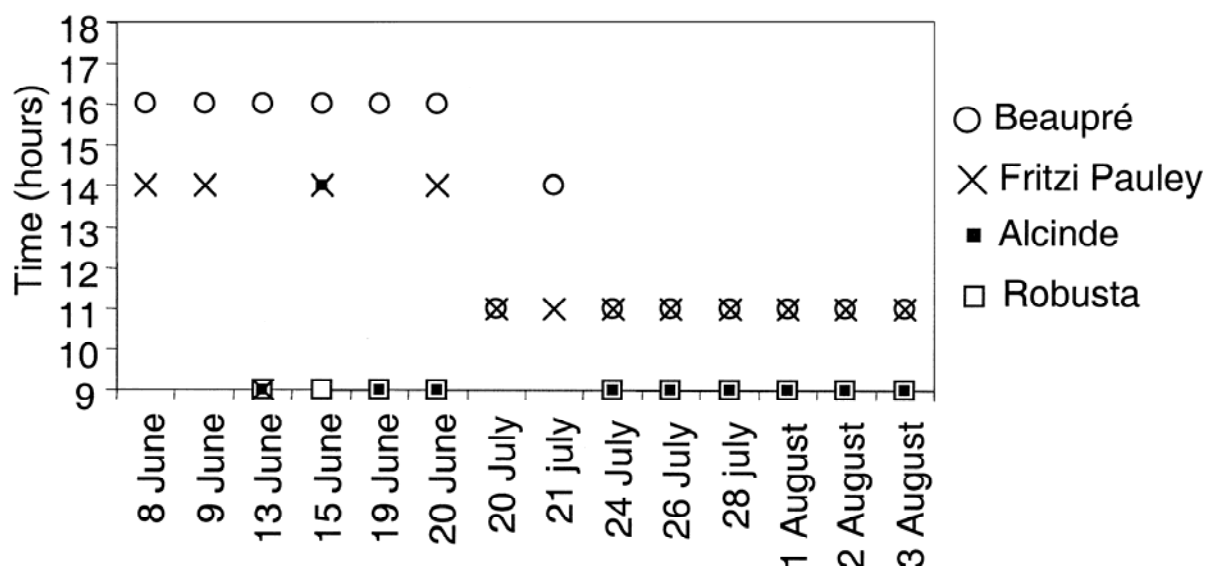


Fig. 5. Persistence of dew in Guémené-Penfao nursery in 2000 on four poplar cultivars.

clones, and 4 (T × D) × T clones. Data from the nursery (infection and dew frequency) were from Nancy in 2003. A significant correlation was found between infection and water droplet contact angle ($r = 0.60$, $P = 0.014$), between infection and drying time in the laboratory ($r = 0.74$, $P = 0.0009$), and between infection and dew frequency in the nursery ($r = 0.69$, $P = 0.003$).

DISCUSSION

Wide variability among poplar species and hybrids was found in the laboratory for water droplet contact angle and drying time, and in the nursery for frequency and persistence of dew. Droplets on black poplars (*P. nigra*, *P. deltoides*, and their hybrids) presented low contact angle values (i.e., flat-shaped droplets) and dried faster. On these clones, dew was less frequent and less persistent. Conversely, water droplet contact angles on balsam poplars and their hybrids were higher, i.e., more spherical droplets that needed more time to dry. A more extensive study of intraspecific variation within the three pure species (*P. nigra*, *P. deltoides*, and *P. trichocarpa*) would be worthwhile.

The relationships found between the characteristics studied in the nursery and laboratory suggest that dew frequency in the nursery will be sufficiently informative and less time-consuming than measuring dew persistence. In the laboratory, drying time of calibrated water droplets also is a valuable shortcut.

The variability, mainly between poplar species and hybrids, described here for water droplet contact angle and dew persistence raises several questions on the determinants of the leaf surface structure. The role of chemical composition of leaf wax, and of microrelief, trichomes, and leaf veins should be explored. Martin and Juniper (14) indicated that poplar wax is made of C26 and C28 2- and 3-methylalkanes, although the quantitative and qualitative variability within the genus *Populus* is unknown. According to Challen (cited in 14), the macroscopic surface roughness, caused by ridges or trichomes or both, is the chief factor limiting the wetting of some species and the superficial wax plays a dominant part in limiting the wetting of others. Holloway (8) gave the following interpretation of contact angle. When less than 90°, roughness is of little significance, and wax, although playing some part in repellence, is not a predominant surface feature. With contact angles in the range 90° to 110°, the leaf surface is probably covered with a smooth layer of wax. Above 110°, the surface is entirely covered by a wax of ultra-microscopic crystalline form. Dew formation and persistence are dependent not only on leaf surface characteristics but also on plant architecture, including leaf size, orientation, and petiole mobility (9). A well-

known feature of black poplars is that they have smaller leaves than balsam poplars and *P. × interamericana*.

Infection in nature, under a given *M. larici-populina* inoculum population, depends on the degree of partial resistance, a feature that is usually assessed in the laboratory without limitations imposed by humidity (i.e., inoculated leaves or leaf disks floating on water in petri dishes). In the laboratory, 2 h of wetness was sufficient to induce some infection by *M. larici-populina*; however, maximum infection was reached after 12 h of moistening, a value in the same range as that reported for other rusts (1, 15, and 20). Our results are in accordance with those of Hamelin et al. (7), who showed that maximum infection of artificially inoculated detached leaves of *P. deltoides* with *M. medusae* f. sp. *deltoidae* occurred after 8 h of continuous leaf wetness. The link described for *P. × interamericana* and its backcrosses with *P. trichocarpa* and *P. deltoides* between infection in the nursery and drying time in the laboratory or water droplet contact angle confirms that the development of *M. larici-populina* is correlated with dew persistence and leaf surface characteristics. Additional evidence, such as segregation of rust incidence in association with wettability in a poplar pedigree, would strengthen this correlation. The finding that backcrossing *P. × interamericana* with *P. deltoides* is correlated with lower dew persistence and with reduced infection by *M. larici-populina* is of special interest to breeders. A type of balsam hybrid recently created by IBW, *P. trichocarpa* × *P. maximowiczii*, presented interestingly short drying time values. In practice, when several clones exhibit the same level of partial resistance in vitro, it is suggested to select those with the shortest time for drying. This last trait has several advantages: it is inexpensive and easy to measure and likely to be under genetic control with a good heritability according to the results after successive backcrosses with *P. deltoides*. With the major problem of pathotype diversity in *M. larici-populina* (19), the characteristics associated with leaf moistening present a significant advantage as they are effective prior to host penetration and to recognition of the pathogen by the plant. Therefore, they are likely to be independent of pathotype variability. Wettability should be a useful tool to be added to the breeder's selection criteria for breeding poplar for durable resistance to *M. larici-populina*.

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