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Components of maritime pine susceptibility to twisting rust – A path coefficient analysis

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Abstract

Data collected in a three-year field experiment on infection and damage by *Melampsora pinitorqua* on maritime pines showing contrasting growth were analysed by means of path analysis. The severity of infection in 2 year-old pines could be mainly explained by the amount of susceptible tissues (elongating shoots). In contrast, two years later, 'intrinsic susceptibility', i.e. expressed after inoculation, was shown to have the most important direct effect on the amount of infection. Intrinsic susceptibility could be satisfactorily assessed by artificial inoculations on excised shoots under controlled conditions. Shoot potassium and phosphorous contents were shown to have marked positive effects on susceptibility. The hypothesis of an age effect on the relative importance of different resistance mechanisms, i.e. avoidance and active resistance, in maritime pine interaction with twisting rust is discussed.

Introduction

Twisting rust, caused by *Melampsora pinitorqua* Rostr., is a common disease of pines in the whole of Europe, mainly on *Pinus silvestris* L. and *P. pinaster* Ait. (Klingstrom, 1963). Infections are caused by airborne basidiospores, disseminated in spring. Basidiopores are produced from teliospores on dead aspen leaves, the alternate host of *M. pinitorqua*. They can infect elongating shoots, on young pines, up to 5–10 years old. Infection sites can be healed, but cessation of growth at this point of the shoot usually results in a typical bending. Weaker shoots are killed, which may result in the formation of several stems. Several studies, either on Scots or maritime pine, have stressed an overall positive correlation between pine susceptibility and vigour (Illy, 1966; Klingstrom, 1969; Jalkanen and Kurkela, 1984; Desprez-Loustau and Baradat, 1991; Baradat and Desprez-Loustau, 1997). This positive relationship has already been mentioned for other biotrophic fungi (Powers et al., 1981; Savary et al., 1988). From a practical point of view, this is an unfavourable relationship, which may lead to increased disease hazard with intensive silviculture or genetic breeding, and thus at least partial loss of potential growth gains. Some results already support this hypothesis (Martinsson, 1985; Desprez-Loustau and Wagner, 1997). A deeper knowledge of components of pine susceptibility to twisting rust, and their relationships with growth traits might form the basis for a more efficient selection and provide indications for improved management practices.

Pine vigour, promoted by silvicultural practices and/or genetic selection for example, may influence susceptibility to *M. pinitorqua* in various ways. A first effect is an increase in susceptible tissues through increased shoot elongation, as succulent shoots are a target for the fungus. Such an effect has also been pointed out for fusiform rust on pines (Hollis and Schmidt, 1977). A second likely effect is a change in tissue quality, resulting from changing patterns of sink-source relationships for nutrients and photosynthates (Loehle and Namkoong, 1987), which may affect both constitutive and induced defenses. Several examples have been reported, mostly with plant-herbivore and plant-insect interactions (Bryant et al., 1983, Ross and Berisford, 1990). More generally, relationships between susceptibility and tissue nutrient content have been demonstrated (Schmidt et al., 1972). Improved growth conditions are also known to accelerate ontogenetical aging (Fortanier and Jonkers, 1976; Kremer and Xu, 1989), which in turn has been shown to have an effect on several host-pathogen interactions, including some rust diseases (Zagory and Libby, 1985; Smit and Parlevliet, 1990). Finally, vigour may not only influence susceptibility, expressed as probability of infection, but also the reaction of trees to infection, and thus damage. Compartmentalization of the fungus and structural resistance of the shoot are likely to be dependent on the pattern of photosynthate allocation to lignin in elongating shoots (Chung and Barnes, 1980).

Within the general frame of investigations about the influence of intensive silviculture on maritime pine health (Jactel et al., 1995), this study was undertaken in order to determine how several traits related to growth (vigour) may be involved in different components of pine susceptibility to twisting rust. We used a multivariate statistical approach with data from a field experiment comparing different silvicultural practices, which resulted in a wide range of variation for pine growth (Desprez-Loustau and Wagner, 1997). As all variables assessed to characterize vigour, such as height, precocity, mineral content, etc., are interdependent, we used path coefficient analysis, a method quite suitable to study the influence of a set of interrelated variables on a variable of interest (Dewey and Lu, 1959; Kingsolver and Schemske, 1991).

Material and methods

Data collection

Data originated from a field experiment previously used to study for the effects of silvicultural practices on pine susceptibility to rust and described in detail (Desprez-Loustau and Wagner, 1997). The experiment was established in 1991, at Castillonville, near Bordeaux, with a local provenance of maritime pine. Eight treatments resulting from the combination of three factors with two levels were compared: planting vs. sowing $*$ 2 initial densities $*$ phosphate fertilised vs. non-fertilised. Treatments were assigned to five blocks, containing 40 unit-plots. Each unit plot had a surface area of approximately 960 m^2 and consisted of three rows of pines, 80 m long and 4 m apart. Each unit plot was edged by a row planted with aspen. Observations on pines were made during three years, 1993–1995, at the age of maximum susceptibility. The following records were made on pines in every third row:

- height, measured during winter,
- receptive shoot length, i.e. the part of the shoot unprotected by budscales, and phenological stage (using a 0 to 5 scale, cf Desprez-Loustau and Dupuis, 1994) of the leader shoot, both at approximately weekly intervals in spring,
- shoot diameter, measured once in April,
- infection of the leader shoot and of lateral shoots in the upper whorl, i.e.; the presence of at least one aecium per shoot, assessed after the infection season in July,
- damage on the leader shoot, scored in autumn (0 = no damage, 1 = slight distortion, 2 = severe distortion),
- final damage, assessed in October 1995, considering the whole bole, with the same 3-level scoring scale as used for the leader shoot.

Basidiospore release experiments and weather data were used to determine the likely period of infections in each year (not shown). This happened to be the period 5–25 April in all three years. The mean values of shoot length and phenological stage were then calculated over the 3–4 records in April for each tree to provide an assessment of these traits at time of infection.

The value calculated for each pine was the proportion of infected shoots related to the total number of shoots in the upper whorl (laterals + leader), which was previously shown to provide the best estimate for susceptibility (Baradat and Desprez-Loustau, 1997).

Additional data were collected in 1995. The whole shoot mineral content was analysed for major nutrients (nitrogen, phosphorous and potassium), and susceptibility was assessed under controlled conditions, using the cut-shoot technique (Desprez-Loustau, 1990) and local *M. pinitorqua* inoculum. Shoots were excised from the upper whorl of pines in May, when most pines presented the B3 or B4 phenological stage. As pines were still monitored for natural infections, shoots were taken in pines of adjacent rows in the same unit plots, one shoot for nutrient analysis and one for inoculation per tree. In 1996, the inoculation experiment was repeated with shoots excised from pines which had been observed for natural infections in the preceeding years.

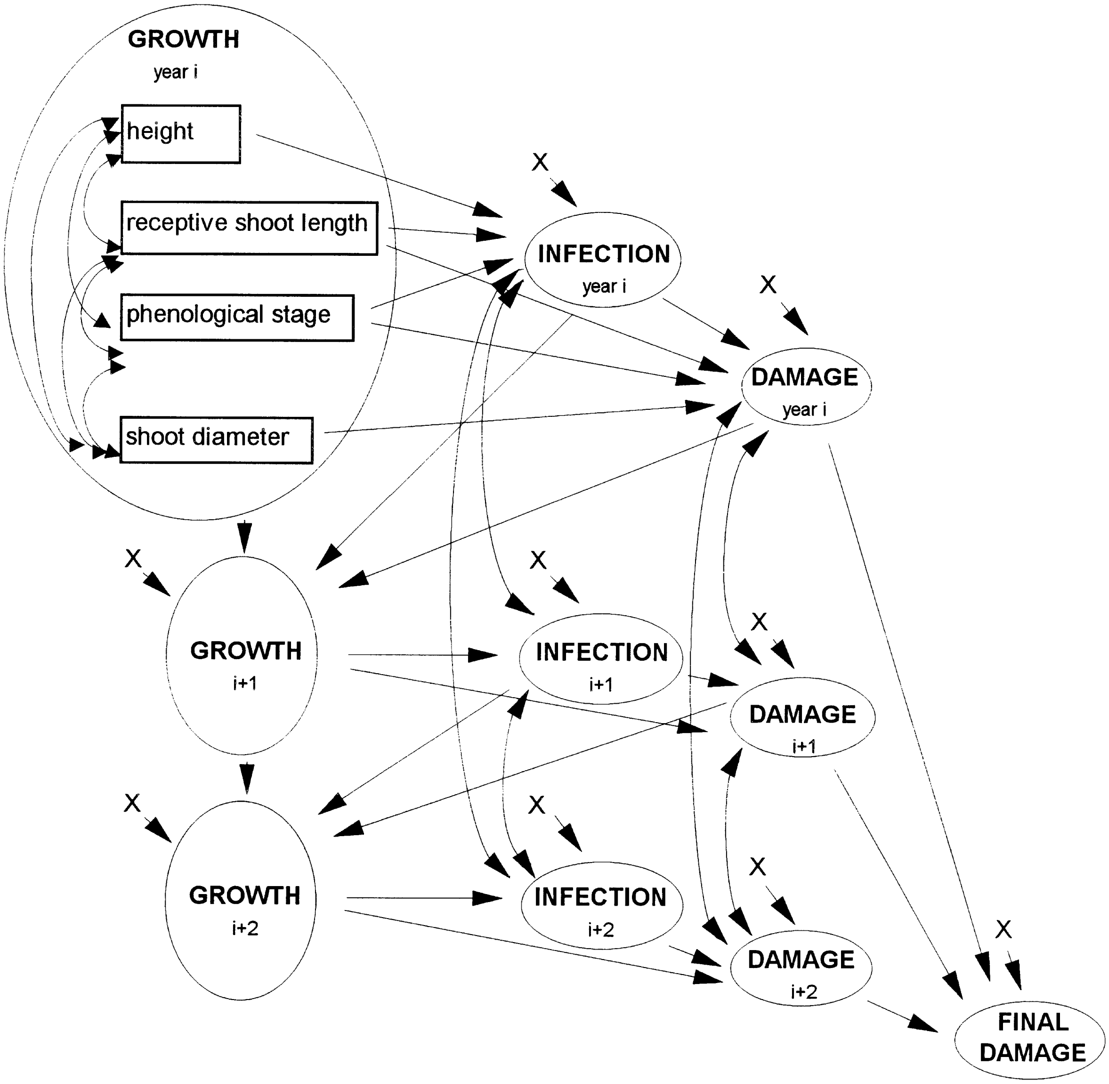

Figure 1. Relational diagram between maritime pine growth variables and *Melampsora pinitorqua* infection and damage: general model for the three-year analysis (Castillonville, 1993–1995). Single-headed arrows indicate direct effects, double-headed arrows represent correlations between variables. X indicate the residual factors for each dependent variable. Growth variables were detailed only for the first year in the diagram.

For both years, inoculations were performed by putting two discs (7 mm diameter) cut out of aspen leaves with abundant germinating telia into contact with each shoot (average inoculum load obtained from 10 pooled discs = 5800 and 5600 basidiospores per disc in 1995 and 1996, respectively). Shoots were then kept overnight in boxes with saturating humidity at 17 °C. After the inoculation period, the incubation took place in a growth chamber at 17 °C, 16h/8h day/night period and approximately $100 \mu E^{*} m^{-2*} s^{-1}$ illumination. Shoots were assessed for the presence/absence of aecia after 3 weeks.

For all variables, the means from unit plots (8 to 15 individuals) were used as the basic data for analysis.

Path analysis

Path analysis, first described by Wright (1921), is based on a path diagram presenting the arrangement of a set of variables interacting with each other and influencing a variable of interest, as hypothesized by the investigator (Dewey and Lu, 1959). Mutual associations are measured by correlation coefficients, causal relationships by path coefficients. The principle of path analysis is a decomposition of the correlation coefficient between each independent variable and the dependent variable into direct and indirect effects according to the paths specified in the diagram. Intermediate dependent variables may be included in the model (Kingsolver and Schemske, 1991). Each analysis with a dependent variable results in a set of n equations with n unknowns (with n the number of independent variables) which can be solved using matrix algebra. The effect of the residual variable is calculated from the part of variation unexplained by the effects of the variables included in the model (Dewey and Lu, 1959). An alternative approach (not used in this study) is multiple regression, with path coefficients being standardized partial regression coefficients.

The relational diagram we hypothesized for pine rust susceptibility is presented in Figure 1.

The environment was assumed to be homogeneous over the experimental field, for abiotic factors, as well as inoculum density due to the regular inter-planting and infection of the alternate host, from which inoculum originates each year. Thus variation in pine infection among elementary plots (considered as individuals, see above) each year was considered to be influenced only by pine phenotype. In addition, not all possible links between measured variables were considered, but those with a biological significance according to previous knowledge on the disease. No direct effects between infection and damage variables recorded in different years were included, since no pine-to-pine infections occur and thus aecia produced in year i-1 do not cause directly infections in year i. Infection and damage in successive years were considered to be linked only by mutual association, likely including their common relationship with genetic resistance, and by indirect effects through growth variables. Thus each year, the variables considered for their direct effect on infection were only growth variables measured in that year.

The epidemiological basis of this diagram is that successful infections, i.e. with sporulation of the fungus (aecia) result from two main processes: (1) inoculations, resulting from the deposition of basidiospores onto the shoot; (2) post-inoculation success or infection ratio, i.e. the percentage of successful infections per 100 basidiospores deposited on the shoot surface.

Height and shoot length were considered as pine traits involved in the probability of inoculation. Under our experimental conditions, the height of trees was a main source of variation for the distance of shoots from inoculum (lying on the ground). The correlation coefficients were calculated directly with height, as linearity was satisfactory. This is also consistent with results from Durrieu (1967), presenting a quasi-linear relationship between pine infection and the distance from *M. pinitorqua* inoculum. Receptive shoot length at time of basidiospore dispersal, as estimated by the length of the shoot unprotected by scales, was used to quantify the amount of susceptible tissues.

Phenological stage at time of infection was considered as likely to be involved in the infection ratio, based on previous studies (Desprez-Loustau, 1990). In the analysis of 1995 data, nitrogen, phosphorous and potassium content were also included. Moreover, susceptibility assessed under controlled conditions – with standardized inoculum put in contact with susceptible tissues – was used as an at least partial estimate of the "intrinsic susceptibility" of shoots. At this point, two competing models were compared. In the first one (Figure 2a), "intrinsic susceptibility" was estimated by the shoot 'qualitative' traits (phenological stage, nutrient concentrations) which were then allowed to influence pine infection directly. In the second one (Figure 2b), susceptibility assessed on excised shoots was considered as an estimate of intrinsic susceptibility and an intermediate variable for shoot qualitative traits through which they may exert an indirect effect on field infection.

Finally, annual damage was considered to be a result of infection and pine reaction to it (Figure 1). Likely components of the latter were considered to be the diameter and length of the shoot, as its distortion or it being killed may be a result of the relative part of injured area to the total shoot area. Phenological stage may also have an effect through ontogenetical changes in lignification. Shoot length and phenological stage were therefore allowed to have both a direct effect on damage and an indirect effect through infection. Final damage was studied as a result of the three subsequent annual damages.

The following path analyses were conducted:

- analysis of susceptibility in the three subsequent years with three morphological growth variables: height, receptive shoot length, phenological stage
- analysis of susceptibility in one year (1995, 4-year-old pines), with morphological growth variables,

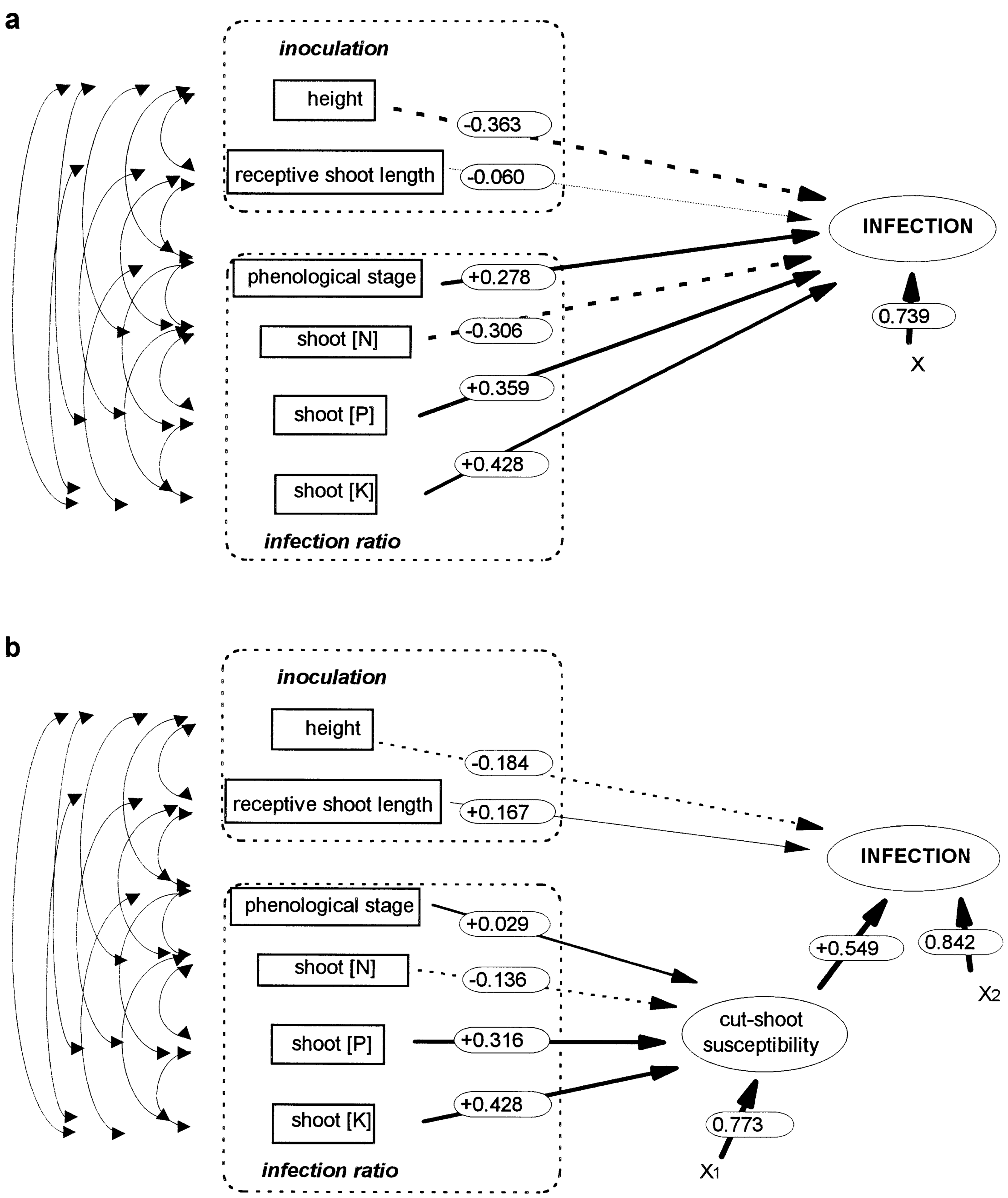

Figure 2. Relational diagram between growth and mineral content variables, and *Melampsora pinitorqua* infection in maritime pines (Castillonville, 1995). Growth traits can affect infection either in the inoculation process (amount of susceptible tissues, assessed by shoot length, and distance from inoculum, assessed by tree height) or in the post-inoculation success or infection ratio. (a) First model with no intermediate variable for the post-inoculation success; (b) Alternative model with susceptibility test data as an estimate of post-inoculation success. Double-headed arrows represent correlations between variables, single-headed arrows indicate direct effects, with solid lines for positive effects and dashed lines for negative effects (the width of the line indicates the magnitude of the effect). X, X1 and X2 are the residual factors for infection (model a), cut-shoot susceptibility and infection (model b), respectively.

Table 1. Mean and standard deviation of growth and *Melampsora pinitorqua* susceptibility variables recorded on maritime pines (Castillonville, 1993–1995)

	H* (cm)	RL (cm)	PS	DIAM (mm)	[N] (%)	[P] (%)	[K] (%)	SUC (%)	PINF (%)	DAM	FDAM
1993	37.7 (14.1)	4.7 (1.5)	2.8 (0.4)	—	—	—	—	—	30.3 (15.4)	0.47 (0.26)	—
1994	89.0 (25.4)	8.6 (2.3)	2.3 (0.3)	5.2 (0.7)	—	—	—	—	21.3 (9.7)	0.55 (0.24)	—
1995	137.9 (37.6)	9.3 (3.7)	2.4 (0.3)	8.3 (1.7)	1.61 (0.19)	0.23 (0.04)	0.95 (0.11)	0.25 (0.16)	19.4 (10.3)	0.18 (0.09)	0.50 (0.30)
1996								0.30 (0.19)			

*Tree Height, Receptive Length, Phenological Stage (0 to 5 scoring), Diameter, Nitrogen content, Phosphorous content and Potassium content of the leader shoot, Susceptibility Under Controlled conditions (proportion of shoots with aecia), Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot), Damage on the leader shoot (0 to 2 scoring), Final Damage for the whole tree (0 to 2 scoring). In each cell, the mean is given on the first line and the standard deviation on the second line in parentheses.

Table 2. Correlation coefficients between annual *Melampsora pinitorqua* infection percentage and morphological growth variables in maritime pines (Castillonville, 1993–1994)

	PINF*	H	RL	PS
PINF		**0.41682**	**0.90593**	**0.71272**
H	0.18266		**0.60889**	–0.03188
RL	**0.32190**	**0.35093**		**0.62236**
PS	–0.03226	–0.25041	**0.47711**	

*Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot), tree Height, Receptive Length and Phenological Stage of the leader shoot.
Upper right diagonal: 1993 data.
Lower left diagonal: 1994 data.
Bold figures indicate significant correlations at the 5% level.

mineral content data and susceptibility assessements under controlled conditions

– analysis of annual rust damage with infection and growth variables (height, receptive shoot length, phenological stage and diameter)

– analysis of final damage with annual damage data

Path analysis of 1995 infection with morphological growth, mineral content and inoculation variables

Finally, relationships between infection and damage variables in successive years were examined. Indirect effects expressed through height and shoot length were studied in more detail, as infection and damage in year i-1 may have a direct effect on these traits in year i. The first one may be easily explained by an effect of infection on further shoot elongation, thus eventually on height. Some reactions to infection, such as crookedness or leader recovery, may directly affect height. The effect of infection or damage in year i-1 on shoot length in year i is explained by the fact that shoot growth in maritime pine is of the fixed type, i.e. with the elongation of preformed units, initiated in the preceding year (Lanner, 1976). Direct effects of infection and damage in year i-1 on height and shoot length in year i were calculated by path analysis, with height and shoot length in year i-1 as the two other explaining variables.

All relationships were checked for linearity and the calculations of path coefficients were made using matrix algebra, with the MathCad software (MathCad, 1994).

Results

Mean values and standard deviation for all studied variables are given in Table 1. The growth of pines from 1993 to 1995 resulted in increased values for height, shoot length and diameter. The natural infection variable followed an opposite trend. Damage was high in the first two years and much less in 1995. The final value was in the same order of magnitude as the former ones (0.5 on average, with a three-grade scale scoring 2 as the highest damage). The coefficients of variation

Table 3. Correlation coefficients of growth and *Melampsora pinitorqua* susceptibility variables in maritime pines (Castillonville, 1995)

1995	PINF*	H	RL	PS	[N]	[P]	[K]	SUC
PINF	1							
H	–0.02389	1						
RL	–0.02549	**0.82757**	1					
PS	0.02793	**0.68889**	**0.89011**	1				
[N]	–0.07212	**–0.63905**	**–0.43461**	**–0.34544**	1			
[P]	**0.50835**	0.08584	–0.10195	–0.09578	–0.03765	1		
[K]	**0.53949**	–0.06961	–0.02006	–0.04294	0.20167	**0.44347**	1	
SUC	**0.52976**	0.04073	–0.07227	–0.04085	–0.07970	**0.46851**	**0.45831**	1

* Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot), tree Height, Receptive Length, Phenological Stage, Nitrogen content, Phosphorous content and Potassium content of the leader shoot, Susceptibility Under Controlled conditions (proportion of shoots with aecia). Bold figures indicate significant correlations at the 5% level.

Table 4. Path coefficient analysis of the relationships between *Melampsora pinitorqua* infection and morphological growth variables in maritime pines (Castillonville, 1993–1995)

Pathways of association	1993	1994	1995
H vs. Infection*			
direct effects	–0.099	–0.047	0.029
indirect effects:			
via RL	0.521	0.163	–0.225
via PS	–0.006	0.067	0.172
Total correlation	0.416	0.182	–0.023
RL vs. Infection			
direct effects	0.856	0.466	–0.272
indirect effects:			
via H	–0.060	–0.017	0.024
via PS	0.110	–0.127	0.223
Total correlation	0.905	0.321	–0.025
PS vs. Infection			
direct effects	0.177	–0.266	0.250
indirect effects:			
via H	0.003	0.012	0.020
via RL	0.533	0.222	–0.242
Total correlation	0.712	–0.032	0.027
Residual	0.374	0.949	0.993
Coefficient of determination	0.86	0.15	0.01

* Infection recorded under natural conditions as the proportion of shoots with aecia in the upper whorl level, including leader shoot. H, RL, PS: tree Height, Receptive Length and Phenological Stage of the leader shoot.

were much higher for susceptibility variables, around 50% for all of them, than for growth variables.

Path analysis of annual infection with morphological growth variables

Correlation coefficients among all variables and path analysis are given in Table 2, 3 and 4. Effects of growth variables on infection differed greatly among variables and years. In 1993, high positive correlation coefficients between the three growth variables and infection were observed and a high coefficient of determination was obtained in path analysis (0.86). Receptive shoot length had both an important direct positive effect on infection and indirect effects, also positive, from height and phenological stage. Conversely, these two variables showed very small direct effects on infection, especially height. Phenological stage had a slight positive direct effect. In 1994, a noticeable though lower direct effect of receptive shoot length was still observed, but residual factors were important to explain differences in pine infection, as shown by the low coefficient of determination (0.15). This was near zero in 1995, with all path coefficients less than 0.3 in absolute value.

Introduction of shoot mineral content data in the model describing 1995 infections (Figure 2a) lead to an important increase in the coefficient of determination, 0.45 as compared to 0.01 with only morphological growth variables (Table 4 and 5). It also gave different estimates of the path coefficients for the first introduced variables, namely height and receptive shoot length, as compared to the incomplete model. In the six-variable model, height showed a marked negative direct effect on infection, while shoot length had none. Indirect negative effects via height were also observed for phenological stage and receptive shoot length, which almost

Table 5. Path coefficient analysis of the relationships between *Melampsora pinitorqua* infection, morphological growth variables and shoot mineral content data in maritime pines (Castillonville, 1995)

	H*	RL	PS	[N]	[P]	[K]	PINF
H	**–0.363**	–0.049	0.191	0.196	0.031	–0.030	–0.024
RL	–0.300	**–0.060**	0.247	0.132	–0.037	–0.009	–0.025
PS	–0.250	–0.053	**0.278**	0.106	–0.034	–0.018	0.028
[N]	0.232	0.026	–0.096	**–0.306**	–0.014	0.086	–0.072
[P]	–0.031	0.006	0.027	0.012	**0.359**	0.190	0.508
[K]	0.025	0.001	–0.012	–0.062	0.159	**0.428**	0.539
residual = 0.739			coefficient of determination = 0.45				

*Tree Height, Receptive Length, Phenological Stage, Nitrogen content, Phosphorous content and Potassium content of the leader shoot, Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot). On each line, the direct and indirect effects on infection of the variables named in the first column are given: the figure in bold characters gives the path coefficient (direct effect), other figures give the indirect effects via other variables, named in the first line. The right column gives the correlation coefficient of each variable with infection.

or more than counterbalanced their respective direct effects. Potassium concentration presented the most important direct effect, in a positive direction, the effect of phosphorous being slightly less. Both had also an indirect positive effect via the other. Nitrogen, on the opposite, had a negative direct effect on infection.

In the alternative model tested, susceptibility assessed under controlled conditions (SUC) was included as an intermediate variable between shoot qualitative traits and infection (Figure 2b). SUC was positively related with 1995 field infection for both 1995 and 1996 inoculation experiments. However, a much tighter relationship was shown for SUC96: r = 0.530 instead of 0.238 for SUC95. Only the former was thus used for subsequent analyses. Nearly the same effects were shown for potassium and phosphorous on SUC than previously on infection (Figure 2). The effect of nitrogen on SUC was twofold less than that of potassium and phosphorous and in the opposite direction. Phenology and major nutrient data accounted for approximately 40% of SUC variation. SUC in turn had the far most important direct effect on infection, in a positive direction. Height had a slight negative effect and shoot length a slight positive one. However, the whole model accounted for only 30% of total variation in 1995 field infection.

Path analysis of annual damage with infection and growth variables

Results are presented in Table 6, with the path diagram presented in Figure 1. Infection was considered as a partly intermediate variable for the influence of shoot length and phenology on damage. As expected, infection had a marked direct positive effect for the 3 years. However, it decreased sharply from 1993 to 1995. In the same time, growth variables increased their relative influence. In 1995, the three morphological growth variables had more important direct effects, whatever the direction, than infection. Moreover, their indirect effects via infection were extremely low. The highest effect was observed for shoot length in a positive direction, whereas phenological stage and diameter had negative effects. Opposite directions were observed in 1994. However, only approximately 25% of damage variation could be accounted for by the model in 1994 and 1995, as compared to more than 60% in 1993. The proportion of explained variation was not increased in 1995 by adding nutrient content data (results not shown).

Path analysis of final damage

Results are presented in Table 7. Damage in 1994 was shown to have the most important part in total damage as compared to the two other years. The three annual damages accounted for more than 60% of total variation in final damage.

Table 6. Path coefficient analysis of the relationships between *Melampsora pinitorqua* damage and morphological growth variables in maritime pines (Castillonville, 1993–1995)

Pathways of association	1993	1994	1995
PINF vs. Damage*			
direct effects	**0.719**	**0.410**	**0.320**
indirect effects:			
via RL	0.208	–0.150	–0.017
via PS	–0.150	–0.010	–0.014
via DIAM		0.141	–0.006
Total correlation	0.777	0.389	0.282
RL vs. Damage			
direct effects	**0.230**	**–0.468**	**0.654**
indirect effects:			
via PINF	0.651	0.132	–0.008
via PS	–0.131	0.153	–0.449
via DIAM		0.339	–0.379
Total correlation	0.750	0.156	–0.182
PS vs. Damage			
direct effects	**–0.210**	**0.320**	**–0.504**
indirect effects:			
via PINF	0.512	–0.013	0.009
via RL	0.143	–0.223	0.582
via DIAM		0.033	–0.339
Total correlation	0.445	0.118	–0.252
DIAM vs. Damage			
direct effects		**0.484**	**–0.464**
indirect effects:			
via PINF		0.119	0.004
via RL		–0.328	0.534
via PS		0.022	–0.368
Total correlation		0.297	–0.294
Residual	0.602	0.856	0.875
Coefficient of determination	0.637	0.268	0.235

Damage assessed on the leader shoot with a three-scoring scale. PINF, RL, PS, DIAM: Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot), Receptive Length, Phenological Stage and DIAMeter of the leader shoot.

Table 7. Path analysis of final *Melampsora pinitorqua* damage in maritime pines with annual damages (Castillonville, 1995–1995)

	DAM93*	DAM94	DAM95	FDAM
DAM93	**0.298**	0.062	–0.031	0.328
DAM94	0.028	**0.644**	0.023	0.695
DAM95	–0.035	0.056	**0.259**	0.281
residual = 0.618	coefficient of determination = 0.618			

*DAM93, DAM94, DAM95: annual damages recorded on the leader shoot in 1993, 1994 and 1995, respectively, with a three-scoring scale. FDAM: final damage assessed on the whole tree with a three-scoring scale. On each line, the direct and indirect effects on final damage of the variables named in the first column are given: the figure in bold characters gives the path coefficient (direct effect), other figures give the indirect effects via the other variables, named in the first line. The right column gives the correlation coefficient of each variable with final damage.

Among year relationships for susceptibility variables

Final damage was significantly (or with a value close to significance) and positively related to all annual susceptibility variables. However, susceptibility assessments were mostly non significantly related among the three different years (Table 8). The exceptions were for infection 1993-infection 1994, but with a negative direction, and damage 1994-infection 1995, with a positive direction.

Infections in the susceptibility test performed in 1996 were highly correlated with 1995 natural infections but the correlation was nil with 1993 and 1994 infections. Susceptibility variables in 1993 were shown to have strong direct effects on 1994 growth variables, in a negative way except for damage on height (Table 9). The most important effect was observed from 1993 infection on 1994 shoot length. Effects of 1994 infection on 1995 growth variables were much lower, but again with a marked negative effect of infection on shoot length in the following year. However, for both years, the most important effect on both height and shoot length was by far that of height in the preceding year.

Discussion

Path analysis is a very helpful method in the study of observational data where multiple causality is involved (Mitchell, 1992). It has been extensively used recently in ecological studies, especially in the analysis of selection (Kingsolver and Schemske, 1991; Petraitis et al., 1996). Some investigations in plant pathology have also relied on this method for the study of environmental factors involved in epidemiology (Bowers et al.,1990), components of resistance (Vail and Marois, 1991; Birhman and Singh, 1995) or the effects of disease on several yield components (VanBruggen and Arneson, 1986; Yang et al., 1992). Causal relationships

Table 8. Correlation coefficients among variables related to *Melampsora pinitorqua* susceptibility in maritime pines in different years (Castillonville, 1993–1995)

	PINF93*	PINF94	PINF95	DAM93	DAM94	DAM95	SUC	FDAM
PINF93	1							
PINF94	**–0.388**	1						
PINF95	0.177	0.085	1					
DAM93	**0.777**	–0.242	–0.078	1				
DAM94	0.209	**0.389**	**0.341**	0.096	1			
DAM95	–0.066	0.161	0.282	–0.119	0.088	1		
SUC	–0.019	–0.011	**0.530**	–0.146	0.135	0.125	1	
FDAM	**0.335**	**0.314**	0.240	**0.328**	**0.695**	0.281	0.189	1

*Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot) and Damage on the leader shoot (0 to 2 scoring), in 1993, 1994 and 1995, respectively, Susceptibility Under Controlled conditions (proportion of shoots with aecia), Final Damage for the whole tree (0 to 2 scoring). Bold figures indicate significant correlations at the 5% level.

cannot be proven, of course, by a statistical analysis and their demonstration need an experimental basis (Petraitis et al., 1996). However, path analysis based on some *a priori* knowledge in the system under study can be a useful tool in hypothesis testing and even improvement of biological hypotheses (Kingsolver and Schemske, 1991; Mitchell, 1992). But path analysis relies on a number of assumptions, most often difficult or impossible to verify (Kingsolver and Schemske, 1991). One of these is that variables are measured without error, for a precise estimate of correlations. In our case, the use of plot means as the basic observations may introduce an error in the estimation of correlation coefficients. Means were chosen both for statistical reasons (in conformity with field experimental design and in order to normalize distribution of semi-discrete variables) and for practical reasons (mineral analyses on bulk samples). Another assumption hardly verified is the absence of correlation between residuals and the remaining variables. For these reasons, and the relatively small sample size, we do not intend to put much emphasis on the numerical values of coefficient in themselves but rather on their direction and relative weight.

A first result of the different path analyses is a somewhat different picture of the relationships between pine growth variables and susceptibility to rust than that provided by the consideration of correlation coefficients alone. For example, phenological stage in 1993 showed a high correlation coefficient with infection but only a minor direct effect, because of an important indirect effect through receptive shoot length (Table 4). Thus the tight relationship between precocity and infection may not rely in this case on a higher susceptibility of more advanced phenological stages but rather on its relationship with a greater amount of susceptible tissues. Another example is given by the shoot length-damage relationship in the same year, explained to a large extent by the indirect effect of shoot length through infection. Thus, important shoot elongation was only a minor factor of increased damage in itself. In contrast, direct effects were sometimes higher than correlation coefficients, such as shoot length with infection in 1994 (Table 4) and height with infection, in absolute value, in 1995 (Table 5). Finally, overall low correlations may mask contrasting noticeable effects working in opposite directions. This was observed for nitrogen content – infection correlation in 1995, which resulted from a negative direct effect counterbalanced by a positive indirect effect through height. The apparent lack of relationship between nitrogen content and infection may thus have been due to the fact that low nitrogen contents, which would favour infection under our experimental conditions, were observed in tall trees, which were more prone to escape infections.

However, a most important observation from these analyses is the dramatic variation among years in the growth variable effects on damage and infection.

A satisfactory model for damage could only be obtained in 1993, with infection as the main explaining variable. The higher effect of residual factors in later years may be due in part to a lower biological significance of the recorded variables. Damage was scored primarily with a practical meaning, thus a single score may correspond to very different types of reaction to infection (bending, leader recovery from a lateral shoot, . . .). These are more likely to occur in well developed shoots, on older trees, than on small

Table 9. Path coefficient analysis of height and shoot length in maritime pines as related to the same variables and *Melampsora pinitorqua* infection and damage in the previous year (Castillonville, 1993–1995)

	H94[*]	RL94	H95	RL95
PINF^{**}_{i-1}	–0.392	–0.470	0.073	–0.129
DAM_{i-1}	0.386	–0.194	–0.082	–0.057
H_{i-1}	0.864	0.528	0.964	0.766
RL_{i-1}	–0.024	0.042	0.100	0.306
Coefficient of determination	0.71	0.35	0.98	0.77

[*] Tree Heights and Receptive Lengths of leader shoots in 1994 and 1995, respectively.

[**] Percentage of INFection under natural conditions (proportion of shoots with aecia in the upper whorl level, including leader shoot), Damage on the leader shoot (0 to 2 scoring), Height and Receptive shoot Length in the previous year. The first line gives the dependent variables, the first column the explaining variables; each figure gives the direct effect from the variable corresponding to their line on the variable corresponding to their column.

shoots of young trees which show more uniform reactions (girdling and withering). This increased complexity of damage may also explain the reduced direct effect of infection on damage in 1994 and 1995 as compared to 1993, and the contrasting effects of shoot length, diameter and phenology on damage in 1994 and 1995.

Analyses on 1993 and 1995 infections can be better compared since models explaining a significant part of variation could be obtained. In 1993, a high coefficient of determination was observed for the model with three morphological growth variables. Receptive shoot length appeared as a key factor in infection with a strong positive direct effect and indirect effects from the two other variables. Thus in this year, susceptibility may be explained in terms of a target effect: the larger the target (= amount of susceptible tissues, i.e. elongating shoots), the higher the probability of infection. Such a target effect has also been reported for fusiform rust of pines (Hollis and Schmidt, 1977). The distance of the target from inoculum, assessed as height, was not shown to play an important part in that year. This may easily be explained by the fact that all pines were still small (Table 1) and height thus did not already appear as a limiting factor. The effect of deposition, or inoculum load, on final infection levels has been documented for several pest- or pathogen- host systems (Alexander, 1989, 1990; Ross and Berisford, 1990; Elmquist et al., 1993; Alfaro et al., 1996). It may be explained by several factors: a higher probability of virulent isolates being put in contact with the host, a chemical “mass effect” from the pathogen favouring penetration (Elmquist et al., 1993), or an inability of the host to provide an appropriate defense response to a “mass attack” (Alfaro et al., 1996). The dramatic decrease of the coefficient of determination between 1993 and 1995, despite the same growth variables being included in the infection model, is not thought to be due to higher experimental errors (see the discussion above about the use of means per plot), as intra-plot coefficients of variation were not increased (results not shown). Thus it can be supposed that residual factors not included in the model, i.e. other than morphological growth variables, played a more important role in 1995 as compared to 1993. A good supporting evidence is provided by the more detailed models of 1995 infection with additional data on nutrient content and shoot susceptibility. Introduction of major nutrient contents in the model raised the coefficient of determination from nearly 0 to 0.45, which suggests the importance of shoot nutrient quality on infection in that year. The positive direct effect of phosphorous on both cut-shoot susceptibility and pine infection is in agreement with experiments on fusiform rust of pines where a significant increase of rust was associated with increased levels of phosphorous, to a greater extent than that expected from only a growth effect (Schmidt et al., 1972). The negative direct effect of nitrogen may seem contradictory with many results showing higher rust damage with higher nitrogen availability. However, most studies have dealt with a global effect of nitrogen on disease, taking into account indirect effect on growth (Agrios, 1988). On the other hand, positive effects of nitrogen fertilisation have been demonstrated on the concentration of secondary metabolites, which may have an inhibitory effect on fungi, such as resin acids in pines (Bjorkman et al., 1991). In this more comprehensive model of 1995 infection, height showed a negative direct effect on infection. This is in accordance with our hypothesis of an increasing distance from inoculum reducing infection through basidiospore deposition. In contrast to 1993, pines in 1995 had reached such a height that this effect became noticeable. A short scale distance-from-inoculum effect on infection has also been demonstrated for smut disease of *Silene alba* (Alexander, 1990; Roche et al., 1995). Finally, the model presented in Figure 2b demonstrated the determinant part of “intrinsic susceptibility”, i.e. expressed after inoculation, in 1995 infections. A path coefficient as high as 0.55 was obtained despite the limitations of the assessment method (using cut-shoots and high but not totally controlled inoculum load). This value is much higher than the residual variable in the model of 1993 infections, where the effect of intrinsic susceptibility could not be studied.

These changes among years in factors explaining infections might explain the lack of significant positive correlation for infection variables between years observed in this study and often reported for twisting rust (Klingstrom, 1969; Kurkela, 1973). In addition, relationships between annual infection variables were shown to include indirect effects through growth variables. The significant negative relationship between 1993 and 1994 infections might be explained to a large extent by an important negative effect of 1993 infection on 1994 receptive shoot length, which itself had a positive effect on 1994 infection. According to our results, active resistance (Burdon, 1987), including genetic resistance, may not always be the decisive factor in the amount of maritime pine infection by *M. pinitorqua*, while avoidance (Burdon, 1987), or "frequency of parasite encounters" (Ennos, 1991), being of major importance in particular years. Such changes in the resistance/avoidance balance may be partially attributable to interactions with external causes, involving pathogen and/or environment. However, it is noteworthy that the most important target effect was observed in our study in the year (1993) with overall highest pine infection, suggesting no greater limitations by external factors. Host traits might also be involved. A combination of avoidance and resistance has already been reported for smut-host systems (Elmquist et al., 1993), such as anther-smut disease of *Silene alba* where the relative importance of the two mechanisms has been shown to vary between plant sexes (Alexander, 1989, 1990). For pine twisting rust, the hypothesis of a change in resistance components related to pine ageing may be put forward. The most obvious effect of age is an increased development. As pines grew from 2 to 4 year-old, they developed longer shoots each year, which may explain why shoot size appeared as a critical factor on infection only in the youngest pines. The converse reasoning may apply for height as a late critical factor related to distance from inoculum. The height effect may also contribute to the decreasing target (i.e. terminal shoot) size effect, as shoots get progressively out of reach of basidiospores. Another effect of age might be an ontogenetical change in susceptibility. Such changes, often referred to as adult- or mature-plant resistance, have been reported for many diseases, especially rust diseases (Zagory and Libby, 1985; Smit and Parlevliet, 1990). The increased involvement of intrinsic susceptibility in older pines in this experiment is consistent with results from a previous study where heritability values for susceptibility to twisting rust under natural conditions were higher in 5 year-old maritime pines than in younger ones (Baradat et Desprez-Loustau, 1997). This "scenario" of maritime pine susceptibility to twisting rust involving, first, avoidance linked to the size, then to the distance (height) of the target (shoots), and an increasing effect of active resistance, may vary, especially in timing, in different situations. It is important to consider that the results in path analysis are essentially dependent on the correlation structure in the studied sample. Genotypic variation in pines, among families selected on growth traits, for example, might induce a different partition of avoidance and resistance mechanisms.

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References

Agrios GN (1988) Plant pathology. Third Edition.Academic Press, New York, 703 pp

Alexander HM (1989) An experimental field study of anther-smut disease of *Silene alba* caused by *Ustilago violacea*: genotypic variation and disease incidence. Evolution 43: 835–47

Alexander HM (1990) Epidemiology of anther-smut infection of *Silene alba* caused by *Ustilago violacea*: patterns of spore deposition and disease incidence. J Ecol 78: 166–79

Alfaro RI, Kiss GK and Janchuk A (1996) Variation in the induced resin response of white spruce *Picea glauca* to attack by *Pissodes strobi*. Can J For Res 26: 967–72

Baradat P and Desprez-Loustau ML (1997) Analyse diallele et integration dans le programme d'amelioration du Pin maritime de la sensibilite a la rouille courbeuse. Ann Sci For 54: 83–106

Birhman RK and Singh BP (1995) Path-coefficient analyses and genetic parameters of the components of field resistance of potatoes to late blight. Ann appl Biol 127: 353–362

Bjorkman C, Larsson S and Gref R (1991) Effects of nitrogen fertilisation on pine needle chemistry and sawfly performance. Oecologia 86: 202–209

Bowers JH, Sonoda RM and Mitchell DJ (1990) Path coefficient analysis of the effect of rainfall variables on the epidemiology of *Phytophthora* blight of pepper caused by *Phytophthora capsici*. Phytopathology 80: 1439–1446

Burdon JJ (1987) Diseases and plant populations. Cambridge University Press, Cambridge, 208pp

Bryant JP, Chapin FS and Klein DR (1983) Carbon / nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40: 357–368

Chung HH and Barnes RL (1980) Photosynthate allocation in Pinus taeda. II Seasonal aspects of photosynthate allocation to different biochemical fractions in shoots. Can J For Res 10: 338–347

Desprez-Loustau ML (1990) A cut-shoot bioassay for assessment of *Pinus pinaster* susceptibility to *Melampsora pinitorqua*. Eur J For Pathol 20: 386–391

Desprez-Loustau ML and Baradat P (1991) Variabilité inter-raciale de la sensibilité à la rouille courbeuse chez le Pin maritime. Ann Sci For 48: 497–511

Desprez-Loustau ML and Dupuis F (1994) Variation in the phenology of shoot elongation between geographic provenances of maritime pine (*Pinus pinaster*) – implications for the synchrony with the phenology of the twisting rust fungus, *Melampsora pinitorqua*. Ann Sci For 51: 553–568

Desprez-Loustau ML and Wagner K (1997) Influence of silvicultural practices on twisting rust infection and damage in maritime pine, as related to growth. Forest Ecol Manag (accepted)

Dewey DR and Lu KH (1959) A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy J 51: 515-518

Durrieu G (1967) Note sur l'epidemiologie de la rouille tordeuse du pin: *Melampsora pinitorqua* (Uredinales). Trav. Labor. For. Toulouse, t. VI, vol. IV, art. II: 1–8

Elmquist T, Liu D, Carlsson U and Giles B (1993) Anther-smut infection in *Silene dioica*: variation in floral morphology and patterns of spore deposition. Oikos 68: 207–216

Ennos R (1991) Ecological genetics of parasitism. In: Genes in ecology, Eds. Berry RJ, Crawford TJ and GM Hewitt. Blackwell Scientific Publications, London, 255–279

Fortanier EJ and Jonkers H (1976) Juvenility and maturity of plants as influenced by their ontogenetical and physiological ageing. Acta Hortic 56: 37–44

Hollis CA and Schmidt RA (1977) Site factors related to fusiform rust incidence in North Florida slash pine plantations. Forest Science 23: 69–77

Jactel H, Desprez-Loustau ML and Lung B (1995) Sylviculture intensive du Pin maritime et risques phytosanitaires. Forêt-Entreprise 105: 45–47

Jalkanen R and Kurkela T (1984) Damage and early height losses caused by *Melampsora pinitorqua* on Scots pine. Folia For 587: 1–15

Kingsolver JG and Schemske DW (1991) Path analyses of selection. Trends Ecol Evol 6(9): 276–280

Klingstrom A (1963) *Melampsora pinitorqua* (Braun) Rostr. - Pine twisting rust. Stud For Suec 6: 1–23

Klingstrom A (1969) *Melampsora pinitorqua* (Braun) Rostr. on progenies of *Pinus silvestris* L. and in relation to growth regulating substances. Stud For Suec 69: 1–76

Kremer A and Xu LA (1989) Relationships between first-season free growth components and later field height growth in maritime pine. Can J For Res 19: 690–699

Kurkela T (1973) Epiphytology of *Melampsora* rusts of Scots pine (*Pinus sylvestris* L.) ans aspen (*Populus tremula* L.). Comm Inst For Fenn 78: 1–22

Lanner RM (1976) Patterns of shoot development in *Pinus* and their relationship to growth potential. In: Tree physiology and yield improvement. Ed. by MGR Cannell and FT Last, Academic Press, London, 223–243

Loehle C and Namkoong G (1987) Constraints on tree breeding: growth trade-offs, growth strategies and defensive investments. For Sci 33: 1089–1097

Martinsson O (1985) The influence of pine twisting rust (*Melampsora pinitorqua*) on growth and development of Scots pine (*Pinus sylvestris*). Eur J For Path 15: 103–110

Mathcad (1994) MathCad 5.0, Guide de l'utilisateur. MathSoft Inc., Cambridge (MA), USA, 524 pp

Mitchell RJ (1992) Testing evolutionary and ecological hypotheses using path analysis and structural equation modelling. Funct Ecol 6: 123–129

Petraitis PS, Dunham AE and Niewiarowski PH (1996) Inferring multiple causality: the limitations of path analysis. Funct Ecol 10: 421–431

Powers HR Jr, Schmidt RA and Snow GA (1981) Current status and management of fusiform rust on southern pines. Ann Rev Phytopathol 19: 353–371

Roche BM, Alexander HM and Maltby AD (1995) Dispersal and disease gradients of anther-smut infections of Silene alba at different life stages. Ecology 76(6): 1863–1871

Ross DW and Berisford CW (1990) Nantucket pine tip moth (*Lepidoptera: Tortricidae*) response to water and nutrient status of loblolly pine. Forest Science 36: 719–733

Savary S, Bosc JP, Noirot M and Zadoks JC (1988) Peanut rust in West Africa: a new component in a multiple pathosystem. Plant Disease 72: 1001–1009

Schmidt RA, Foxe MJ, Hollis CA and Smith WH (1972) Effect of N, P, K on the incidence of fusiform rust galls on greenhouse-grown seedlings of slash pine. Phytopathology 62: 788

Smit G and Parlevliet JE (1990) Mature plant resistance of barley to barley leaf rust, another type of resistance. Euphytica 50: 159–162

Vail ME and Marois JJ (1991) Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. Phytopathology 81: 188–191

VanBruggen AHC and Arneson PA (1986) Path coefficient analysis of effects of *Rhizoctonia solani* on growth and development of dry beans. Phytopathology 76: 874–878

Wright SC 1921. Correlation and causation. J Agric Res 20: 557–585

Yang XB, Dowler WM, Tschantz AT and Wang TC (1992) Comparing the effects of rust on plot yield, plant yield, yield components, and vegetative parts of soybean. J. Phytopathology 136: 46–56

Zagory D and Libby WJ (1985) Maturation-related resistance of *Pinus radiata* to western gall-rust. Phytopathology: 75: 1443-1447