The Influence of Temperature and Light on Defoliation Levels of Elm by Dutch Elm Disease

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ABSTRACT

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The amount of defoliation of elm (*Ulmus procera*) caused by three *Ophiostoma novo-ulmi* Eurasian race isolates over 14 seasons of field trials was found to be strongly correlated with mean air temperature and mean number of sunshine hours over the 12-week period from inoculation to assessment, and with tree age. The coefficient of determination

for the regression of percent defoliation on the environmental and tree factors was 0.76, $P < 0.001$ (33 df). Levels of defoliation were greatest when mean air temperatures exceeded 17°C with moderate light (5 to 7 h of sunshine), and lowest under conditions of either high light (>7.5 h of sunshine) at all air temperatures or low light (<4.5 h of sunshine) and air temperatures of less than 15.5°C. The model varied in its intercept for the three isolates, reflecting their different levels of aggressiveness. The role of environmental factors in the development of Dutch elm disease symptoms and the implications for elm resistance breeding are discussed.

The highly destructive vascular wilt disease of elms, Dutch elm disease, is spread from infected to healthy trees by elm bark beetles of the genus *Scolytus* or, more rarely, in North America by *Hylurgopinus*. Two pandemics of this disease have occurred this century: the first caused by *Ophiostoma ulmi* (Buisman) Nannf., and the second and current pandemic by the more aggressive *Ophiostoma novo-ulmi* Brasier (12). *O. novo-ulmi* occurs as two biotypes termed the North American and the Eurasian races, exhibiting partial reproductive isolation and distinguished by their cultural and molecular characteristics (9,11,12,26). The Eurasian race shows greater variation in aggressiveness than does the race from North America (9,10).

The spread of a Dutch elm disease epidemic involves complex interactions between the elm population (20,21), the pathogen (4,7,14,22), the beetle vectors (1,29,31,32,56,58), d-factors ('virus-like' agents of the pathogen) (8), and environmental conditions (21). Although environmental conditions have not been shown to significantly influence the outcome of Dutch elm disease epidemics (20), they have been shown to affect beetle behavior (19) and the efficiency of beetle-mediated disease transmission (56). Environmental factors affect symptom development, both the external levels of defoliation and the extent of internal vascular browning (43,45,46, 49,50), in elms following artificial inoculation, although, as with some other vascular pathogens (3), these two aspects of disease may not be directly linked (24). Kais et al. (28) found that soil type and soil moisture significantly affected the extent of vascular browning in highly susceptible *Ulmus americana* seedlings following inoculation with *O. novo-ulmi*; elms under moisture stress having less vascular browning than nonstressed trees. Birkholz-Lambrecht et al. (5) found that ambient temperature significantly affected the amount of vascular browning in *U. americana* seedlings inoculated with *O. ulmi*, levels being greatest at 26°C. Subsequently, Green et al. (23) and Smalley and Guries (44) reported similar temperature effects with a range of other elm clones.

Wilting and defoliation levels are the main parameters used for assessing elms for resistance in elm breeding programs and in comparative pathogenicity tests of *O. ulmi* and *O. novo-ulmi* isolates (4,9,10,24,44,54). However, they have been found to vary considerably between seasons (43,45,46,49,50). Brasier (10) suggested that this variation reflected first the interactions between the host and environment prior to inoculation (environmental preconditioning), and second the interactions between the host, pathogen, and environment during and after inoculation. The objective of the current paper was to quantify the relative influence of five environmental factors: mean air temperature, mean number of sunshine hours, mean vapor pressure deficit, mean rainfall, and one host factor, tree age, on external defoliation levels in *U. procera* Salisb. resulting from inoculation of three Eurasian race *O. novo-ulmi* control isolates of differing levels of aggressiveness over 14 seasons of field experiments.

MATERIALS AND METHODS

Pathogen isolates. Eurasian race isolates of *O. novo-ulmi* with 'high' (H327), 'medium' (H413a), and 'low' (AST-27) (10) aggressiveness were used in field inoculations; the isolates originated from Czechoslovakia, Germany, and Iran, respectively (7). H327 and H413a represent the common limits of aggressiveness observed in Eurasian race isolates (9), whereas AST-27 is an unusually weak pathogen.

Stock cultures were maintained on 2% Oxoid malt extract agar (MEA) (Unipath Limited, Basingstoke, Hampshire, United Kingdom) plates at 20 to 22°C in the dark and subcultured at 3-week intervals. Long-term stock cultures were maintained on 2% MEA slopes at -20°C and in liquid nitrogen. Before use in annual experiments, the growth rates and colony morphologies of the isolates were routinely examined using the method given by Brasier (6). Mean daily radial growth rates were calculated from colony diameters measured after 2 and 7 days of growth on 2% MEA at 20°C in the dark, and colony morphologies (6,9) were assessed after a further 10 days in diffuse light. No systematic differences were found over the period of study. Only subcultures with the typical morphology of the Eurasian race wild type (6,7,9) were used in the inoculations.

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Conidial suspensions of the isolates were prepared by inoculating 10 ml of Tchernoff's liquid medium (52) in sterile universal bottles with 2 × 2-mm plugs from the edge of 7-day-old cultures on MEA, followed by shaking these liquid cultures for 3 days at room temperature. This produced suspensions of approximately 1 × 10⁶ spores ml⁻¹.

Elm material and inoculation method. Isolates H327, H413a, and AST-27 were used routinely as controls in annual pathogenicity trials of *O. ulmi* and *O. novo-ulmi* isolates or their progenies (9,10,14) conducted between 1979 and 1995. The trials were carried out at the Forestry Commission Experimental Nursery, Headley, Hampshire, United Kingdom, and commonly involved 600 to 1,000 4- to 7-year-old trees per annum. The trial years, tree ages, and number of replicate trees used per control isolate are summarized in Table 1. The trial site was subdivided into 24 plots, each with 120 to 250 elms planted at different dates. Approximately half of the plots comprised *U. procera* clone SR4 propagated from soft-wood cuttings, planted at 1-m spacing. Each year, one of the plots of older (2- to 4-m tall) *U. procera* was used in a pathogenicity trial involving H327, H413a, and AST-27 as controls. Pathogenicity trials were not conducted in 1980, 1986, and 1989, because of shortages of host material or other factors. In 1990 and 1993, trials were conducted on two plots of elms of differing tree ages.

Trees within the plot were categorized according to height, and only similar-sized trees were used. Three to eight replicates of each isolate were applied to trees selected at random within the plot. On completion of the experiment, the trees and roots were removed and destroyed and, in the following spring, the plot was replanted. All inoculations were conducted in mid-June using the method of Gibbs and Brasier (22). A cut was made into the new sapwood one-fifth of the way down the crown of the tree with a flame-sterilized scalpel blade. Two drops of a 1 × 10⁶ spores ml⁻¹ suspension were then applied from a hypodermic syringe; this inoculum was drawn into the xylem immediately. Twelve weeks after inoculation, each tree was assessed for disease in terms of percent defoliation of the crown using three independent assessors. Defoliation was assessed at 1% intervals in the 0 to 20% and 80 to 100% ranges, and at 5% intervals in the 20 to 80% range. The mean of the three assessments was then calculated for each tree (9).

Soil conditions. The soil at the site was greensand and was cultivated every 5 to 6 years to approximately 20 cm and amended annually by organic matter to enhance moisture retention. Plots were subject to nighttime overhead irrigation as required to maintain a soil matric potential of approximately -14 kPa.

Meteorological data. This was collected at the Forest Research Station, Farnham, Surrey, United Kingdom (DCNN 5694, 115.2 m above sea level; Ordnance Survey Map grid reference: SU

804427), 6 km from the experimental site. The data available included daily records of maximum and minimum air temperature, wet and dry bulb temperatures recorded at 9:00 a.m., daily hours of sunshine, and daily rainfall (millimeters). Hourly values of air temperature and irradiance (watts per square meter) for the Forest Research Station and experimental sites were compared during the period of 15 June to 31 August 1995, using matched temperature sensors and pyranometers positioned adjacent to the experimental plots and the meteorological site. Differences between the two sites averaged over the period were small and not statistically significant. For example, at the Forest Research Station, the mean hourly air temperatures were 0.25°C warmer, whereas the mean hourly irradiance was 3.8% greater at the experimental site.

Statistical analysis. Percent defoliation was modeled using multiple linear regression with the SAS statistical package (SAS Institute, Cary, NC). The analysis was performed using combined mean defoliation data for the three isolates studied. Mean defoliation data for different-aged trees within a year were also included. A logit transformation was applied to the proportional defoliation data, and the following independent variables were considered to be explanatory: *O. novo-ulmi* isolate (considered as a factor in the regression), mean air temperature [(minimum + maximum)/2] (*T*), mean number of sunshine hours (*S*), mean vapor pressure deficit (*V_d*) estimated in kilopascals from wet and dry bulb temperatures measured at 9:00 a.m. each day (using the equations given by Monteith and Unsworth [36]), mean rainfall in millimeters (*R*), and tree age in years (*A*). Means for each variate were determined from the data gathered over the duration of the experimental period for each year. A correlation matrix between recorded environmental and tree factors was determined.

Percent defoliation was modeled using a two-stage process. Firstly, a model was constructed containing a maximum number of explanatory factors, interactions, and covariates, all of which were considered to have a possible effect on defoliation levels. Secondly, using the principle of parsimony, the model was reduced to a minimal adequate model by removing parameters that did not lead to significant increases in residual deviance and keeping basic parameters when there was a choice between discarding those or more complex interactions.

RESULTS

Significant differences were observed in the amount of defoliation caused by the three isolates, both over the 14-year experimental period (*P* < 0.05) and within each season (*P* < 0.05), with the exception of 1993 (Fig. 1). Isolate H327 was consistently the most aggressive, causing a mean defoliation of 63% over the whole trial

TABLE 1. Tree age and number of single-tree replicates of *Ulmus procera* inoculated with each of three isolates of the Eurasian race *Ophiostoma novo-ulmi* in field trials between 1979 and 1995 in Hampshire, England

Year of inoculation	Tree age (years)	No. of replicates of <i>O. novo-ulmi</i> isolate		
		H327	H413a	AST-27
1979	6	...	...	3
1981	5	...	...	3
1982	4	...	...	3
1983	5	3	3	3
1984	5	4	6	6
1985	5	6	6	6
1987	5	4	4	4
1988	5	4	...	4
1990	5 and 6	4	4	4
1991	7	8	4	8
1992	5	4	4	4
1993	6 and 7	4	4	4
1994	4	4	4	4
1995	5	4	4	4

^a Not inoculated.

Fig. 1. Levels of mean percent defoliation on *Ulmus procera* following artificial inoculation with three *Ophiostoma novo-ulmi* isolates, H327 (▲), H413a (■), and AST-27 (●) between 1979 and 1995. Shapes of open symbols in 1990 and 1993 correspond to isolates represented by closed symbols, but on younger trees. Bars indicate 95% confidence limits.

period. Moderate levels of defoliation, averaging 43%, occurred with isolate H413a. Isolate AST-27 consistently caused the least disease, with a mean defoliation of 35%. The ranking of the isolates in terms of aggressiveness remained consistent over all trial years.

The correlation matrix among the recorded environmental and tree factors (Table 2) showed that only air temperature and vapor pressure deficit were significantly correlated ($P < 0.05$). Relationships between all other independent variables were not significant.

Regression analysis indicated that a minimal explanatory model should include terms for each isolate of *O. novo-ulmi*, tree age, mean air temperature, and mean number of sunshine hours (Table 3) as described by the following equation, which incorporated a logit transformation:

$$\text{proportional defoliation} = \frac{e^{x_i B}}{1 + e^{x_i B}}$$

in which x = vector of explanatory variables and B = vector of parameters. Also, $x_i B = B_{\text{isolate } j} + B_2 X_{i2} + B_3 X_{i2}^2 + B_4 X_{i3} + B_5 X_{i3}^2 +$

$B_6 X_{i4}$. X_{i2} is the mean number of sunshine hours for tree i , X_{i3} is the mean air temperature for tree i , and X_{i4} is the age of tree i . The analysis confirmed that the isolates differed significantly in terms of their ability to cause defoliation. However, there were no significant interactions between the isolates and any of the other independent variables. Thus, the differences between the isolates were consistent regardless of the prevailing environmental conditions.

Defoliation decreased significantly with tree age, increased quadratically with mean air temperature, and was quadratically related to the mean number of sunshine hours, with no interaction between the variates. The final model (Table 3) showed that there was no significant effect of rainfall or vapor pressure deficit. Overall, the model accounted for 76% of the variation ($P < 0.001$, 33 df) in defoliation between isolates and experimental years.

Using the final fitted model (Table 3), response surfaces were plotted to explore the relationship between defoliation and mean air temperature and mean number of sunshine hours for 5-year-old elms (Fig. 2A to C). To prevent inadvertent extrapolation beyond the data used to fit the model, the rarely observed combinations of higher temperature and lower light, and lower temperature and higher light, were excluded from the regression surfaces. Moreover, in an analysis of meteorological data gathered from the Forest Research Station site since 1949, no combinations of air temperature and sunshine hours lying outside the domain of the regression surfaces had ever occurred.

For all three isolates, the response surfaces indicated that the greatest defoliation occurred when mean air temperatures exceeded 17°C with moderate light levels (5 to 7 h of sunshine). Overall, as air temperature decreased, the level of defoliation was reduced. Below 17°C, defoliation was highest under moderate light levels of 5 to 6 h of sunshine. The response surfaces also demonstrate the differences between the isolates, H327 being consistently the most aggressive isolate and AST-27 the least aggressive isolate.

TABLE 2. Correlation matrix and levels of significance among independent variables used in the multiple regression analysis

Variable ^a	A	V _d	R	S	T
A		0.022	-0.013	0.100	-0.141
V _d			-0.489	0.413	0.595 ^b
R				-0.532	-0.465
S					0.425
T					

^a A is tree age (years) at the time of inoculation; V_d is the mean vapor pressure deficit (kilopascals) for the 12-week period from inoculation to assessment; R is the mean rainfall (millimeters) for the 12-week period from inoculation to assessment; S is the mean number of sunshine hours for the 12-week period from inoculation to assessment; and T is the mean air temperature (°C) for the 12-week period from inoculation to assessment.

^b Significant difference at $P \leq 0.05$.

TABLE 3. Multiple regression of proportional defoliation of *Ulmus procera* caused by Dutch elm disease on environmental variables and pathogen isolates for data collected on 4- to 7-year-old trees over a 14-year period in Farnham, England

Term	Coefficient	F ^a	df	P ^b	Percent variation explained ^c
Included terms					
Constant	21.1				
Isolate					
H327	0.807				
AST-27	-0.649	10.88	2	<0.001***	36.3
H413a	0.0				
Air temperature					
Linear	-4.88	5.30	1	<0.05*	3.6
Quadratic	0.1644	5.84	1	<0.05*	4.2
Sunshine hours					
Linear	6.16	13.87	1	<0.001***	15.5
Quadratic	-0.548	15.71	1	<0.001***	19.6
Tree age	-0.396	10.42	1	<0.01**	9.7
Rejected terms					
Vapor pressure deficit					
Linear		2.21	1	>0.1	
Quadratic		2.60	1	>0.1	
Rainfall					
Linear		2.86	1	>0.1	
Quadratic		2.96	1	>0.05	
Tree age					
Quadratic		0.28	1	>0.5	
Isolate × air temperature		3.23	2	>0.05	
Isolate × sunshine hours		0.88	2	>0.1	
Isolate × tree age		0.03	2	>0.5	
Air temperature × sunshine hours		0.12	1	>0.5	

^a F values refer to dropping the term from the full model, in the case of terms included in the model, and adding the term to the full model, in the case of rejected terms.

^b Asterisks denote significant differences at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

^c Percent variation explained refers to the change in percent of the variance explained by the model if the test term is dropped. Response variate: logit (defoliation level), $n = 41$, and 75.7% of variance explained.

DISCUSSION

Proportional defoliation occurring in elms inoculated with the Dutch elm disease pathogen *O. novo-ulmi* was strongly correlated with two environmental factors, air temperature and sunshine hours, and with one host factor, tree age. The results confirm and quantify some of the wide seasonal differences in symptom development reported previously (10,43,45,46,49,50). Vapor pressure deficit was not found to affect defoliation levels, although it is known to be a critical factor in the entry of the fungus into healthy elms via beetle feeding wounds, the natural infection court (57). Similarly, rainfall was not found to be a significant factor, but its influence was probably largely eliminated since the elms were maintained at a minimum matric potential by irrigation. The overall sensitivity of the analysis was enhanced by the use of a single clone of *U. procera* and the relatively long data series for the three isolates of *O. novo-ulmi* investigated. Although, as expected, *O. novo-ulmi* isolates H327, H413a, and AST-27 differed significantly in their aggressiveness to *U. procera*, the defoliation levels observed were explained by the same basic model for each isolate, with no interaction terms.

The current analysis has shown that high levels of defoliation resulted when air temperatures exceeded 17°C with moderate sunshine exposure (5 to 7 h), whereas low levels of defoliation were associated with either high light (>7.5 h of sunshine) at all air temperatures, or low light (<4.5 h of sunshine) and cooler air temperatures (<15.5°C). Temperature and light have been shown to influence disease development in other vascular pathogens (3,27,55). Both photoperiod and solar radiation affect *Verticillium* wilts of tomato (27) and potato (55), symptom development being more rapid and severe in short-day rather than long-day growing conditions. Ben-Yephet and Shtienberg (3) found that severe epidemics of *Fusarium oxysporum* f. sp. *dianthi* developed at low radiation intensities and temperatures of 25 to 26°C, whereas plants remained symptomless at relatively high radiation intensities and temperatures near 18°C.

Little is known at present of the influence of environmental factors such as temperature and light on the physiology of the elm in relation to its ability to respond to infection, or of their influence on pathogen development or the host-pathogen interaction. A number of host-related factors are known to affect the susceptibility of elms to *O. novo-ulmi*. These include xylem vessel length, vessel diameter, and rates of tree growth (16,35,37,41,42,59); rates of production of vascular occlusions, lignified zones, and tyloses (17,18,33); and rates of production of fungitoxic substances such as mansonones (15,25). In addition, rates of elm transpiration (34,38,39,40), respiration (30), photosynthesis (39), and carbon partitioning alter significantly as disease develops, but whether these alterations reflect the active response of the host to the pathogen or whether they correlate with external symptom development is unknown. Pathogen growth rate and metabolite production are also likely to be affected by conditions within the xylem. A previous analysis demonstrated a strong positive relationship between growth rate (biomass production) of *O. novo-ulmi* in culture and defoliation levels in *U. procera* following inoculation (14). The temperature for optimal growth and biomass production of *O. novo-ulmi* isolates on MEA in culture is 20 to 22°C, and growth rate falls very rapidly above 27.5°C (9,13). The production by *O. novo-ulmi* of cerato-ulmin, an extracellular hydrophobin implicated as a toxin in the wilt syndrome (48,51), also is positively correlated with biomass (47); the optimum temperature for its production being close to its growth optimum (53).

It is important to note that, as with some other vascular pathogens (3), levels of external symptoms such as defoliation do not always reflect the extent of internal symptom development by *O. novo-ulmi*. For example, in 1990, a season with high temperatures and high sunshine hours, defoliation levels were exceptionally low (Fig. 1). However, when the inoculated trees were destructively

sampled at the end of the experimental period, most (92%) were found to have extensive vascular browning throughout the length of the crown. Similar results were observed under high temperature and sunshine conditions in 1995 in another experiment on the moderately resistant *U. × hollandica* cv. 'Commelin', when again

Fig. 2. Response surfaces for 5-year-old *Ulmus procera* showing the relationship between predicted percent defoliation and mean air temperature and mean number of sunshine hours for *Ophiostoma novo-ulmi* isolates **A**, H327; **B**, H413a; and **C**, AST-27. Response surfaces were produced using the multiple regression model in Table 3. The level of percent defoliation in each grid cell was determined by the mean value for the four corners. The grid cell was then assigned the pattern for the level of percent defoliation closest to the calculated mean. 0 to 19%, 20 to 39%, 40 to 59%, 60 to 79%, and 80 to 100%.

most trees had low levels of defoliation (<20%) yet extensive vascular browning throughout the crown (>80%). Possibly, high temperature and light conditions induce stomatal closure leading to restricted internal water movement, whereas downward spread of the pathogen in the xylem is still facilitated. It is already widely recognized that *O. novo-ulmi* somehow moves down the tree 'against the sapstream' (2). Clearly, external symptom expression and internal colonization are not necessarily coupled. The relationship between these two aspects of disease requires more detailed investigation. Nevertheless, levels of defoliation continue to be the primary assessment criteria in elm resistance and fungal pathogenicity trials (4,9,10,24,44,54).

Owing to the enormous numbers of elms lost to Dutch elm disease across the northern hemisphere, considerable effort is being put into breeding disease-resistant elms (24,44,54) and investigating the genetic control of pathogenicity in the fungus (4,9,10), with foliar symptoms as the main criterion for disease assessment. The extent to which environmental conditions cause defoliation levels to vary between years highlights the importance of conducting resistance trials over several seasons, and the value of using well-defined control isolates in pathogenicity tests. The current results also have confirmed the observation of Smalley and Kais (45) that a difference of 1 or 2 years in tree age significantly affects the level of defoliation, tree susceptibility declining with age. It is, therefore, desirable to use older trees of similar age in comparative resistance or pathogenicity studies.

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