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Considerations for Throughfall Chemistry Sample-Size Determination

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ABSTRACT. Both the number of trees sampled per species and the number of sampling points under each tree are important throughfall sampling considerations. Chemical loadings obtained from an urban throughfall study were used to evaluate the relative importance of both of these sampling factors in tests for determining species' differences. Power curves for detecting differences among species derived from the noncentrality parameter developed herein indicated that the number of trees sampled per species affects power more than the number of points sampled under each tree. FOR. SCI. 35(1):173–182.

ADDITIONAL KEY WORDS. Power, noncentrality parameter.

THROUGHFALL STUDIES ARE HELPFUL IN EVALUATING NUTRIENT CYCLING and canopy exchange processes. However, to accurately estimate these processes, a sufficient number of throughfall samples is needed. Results, estimations, and theories of canopy processes may be erroneous without sufficient samples.

Several studies using different statistical methods have estimated the numbers of samples and methods (gage type, roving versus permanent gages, etc.) required for sampling throughfall in natural forests (Helvey and Patric 1965, Kimmins 1973, Mahendrappa and Kingston 1982). However, most of these studies deal with throughfall quantity rather than quality. Very little is known about urban throughfall chemistry, but interest is increasing. The reasons for this new interest are many, including the realization of the importance of understanding urban nutrient cycling and dynamics, and the concern that acid precipitation and atmospheric pollutants may be harming urban vegetation.

Accurate sampling of urban throughfall chemistry is as essential as accurate sampling in natural forests. Toward this end, data that had been collected for an urban throughfall study are used in this paper to evaluate throughfall sampling methodology.

BACKGROUND

Throughfall was sampled under isolated urban trees for three storms during the summer of 1982. Three trees each of sugar maple (*Acer saccharum*

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Marsh.), pin oak (*Quercus palustris* Muenchh.), and London plane (*Platanus acerifolia* Willd.) were used. For the original study, the area beneath each tree was stratified into three circular zones with the base of the tree bole as the center of each zone. The inner zone extended 0.91 m from the tree bole, the middle zone extended from 0.91 m to 1.8 m, and the outer zone extended from 1.8 m to 2.7 m. Within the inner, middle, and outer zones, respectively, 2, 5, and 8 points were sampled randomly; 15 samples were taken under each tree, and 135 throughfall samples were collected for each storm event. Zonal stratification was performed for spatial trend analysis of throughfall chemistry with distance from the tree bole.

Throughfall was collected using 2-liter polyethylene bottles with a 19-cm diameter polyethylene funnel inserted into each bottle. Clean bottles and funnels were placed beneath the canopy prior to each storm event. The same points were sampled under each tree for each of the three storms. Incident precipitation also was sampled at one point central to the study trees, using the same collection apparatus.

When canopy dripping ceased, the samples were retrieved from the field and chemically analyzed with an Orion 901 Microprocessor Ionalyzer in combination with appropriate specific ion electrodes.[1] Chemical analyses were performed for ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N), calcium (Ca), sodium (Na), potassium (K), chloride (Cl), and pH, though Cl was not statistically analyzed because it was rarely detectable in the throughfall samples. A more detailed account of the sampling scheme and methods and materials used for this study is given in Edwards (1983) and Edwards et al. (1983).

All concentrations (mg liter^{-1}) were transformed to loadings (mg m^{-2}) for statistical analyses using:

$$L_i = VC_iQ/A$$

where

$$\begin{aligned} L_i &= \text{loadings for chemical constituent } i, \text{ mg m}^{-2} \\ V &= \text{sample volume, liter} \\ C_i &= \text{concentration of constituent } i, \text{ mg liter}^{-1} \\ Q &= 1 \times 10^4, \text{ cm}^2\text{ m}^{-2} \\ A &= \text{funnel area, cm}^2 \end{aligned}$$

pH was converted to hydrogen ion concentrations prior to the conversion to loadings.

In the original analysis of variance, each rain event was analyzed individually. The sources of variation were species, trees nested within species, zone, and points nested within zone. Interaction terms were found to be nonsignificant, so they were pooled into the error term.

No significant zonal variations were found for NH_3-N, NO_3-N, Ca, Na, or K. Hydrogen did exhibit slightly greater loadings near the crown edges than near the tree boles; however, these differences are believed to have been due more to windblown precipitation entering the outer collectors than to actual throughfall variations. Species differences were generally nonsignifi-

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[1] Orion Research Inc., 380 Putnam Ave., Cambridge, MA 02132. The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

cant, while trees within species differences were frequently significant. The sample loadings under each tree were also greatly variable. Points close to one another often had more dissimilar nutrient loads than points far from one another.

The dissimilarities found among trees within a species and among points beneath a tree led to a question of sampling. Specifically, how do the number of points sampled per tree and the number of trees per species sampled affect the accuracy of throughfall chemistry measurements? To evaluate these variables, the statistical model used in the original experiment was changed to one that could directly address these two sampling considerations. A model was developed that permitted isolation of the two variables in question (i.e., the number of points per tree and the number of trees per species) so that their influences on the precision of estimates and the consequent power of the analysis of variance F-tests could be evaluated.

STATISTICAL DESIGN

Zone was not significant in the original analysis of variance (Edwards 1983, Edwards et al. 1983), so this effect was removed in the new analysis of variance (Table 1) for simplification. Also, the source event was added to the new model since all of the data for all three storms were used in the analysis for this paper, rather than analyzing storms separately. The storm data were combined because individual storm characteristics have less influence on nutrient cycling, foliar nutrient deficiencies, foliar damage, etc. than do cumulative storm effects.

Evaluation of the expected mean squares given in Table 1 shows that only event and species main effects contain terms for both the number of trees within species, J, and the number of points sampled under each tree, M. Thus, the impact that both J and M have on the power can be examined for event and/or species F-tests. However, only the species effect will be examined here.

The power of the F-test is a function of the noncentrality parameter, λ. The relationship of the number of trees within species, J, and the number of points sampled under each tree, M, to λ is demonstrated below.

For the new analysis of variance, in terms of mean squares, the expected value of the F-ratio for the test of species differences has the general form

TABLE 1. Analysis of variance table for the new model.

Source	Abbreviation[a]	Degrees of freedom	Expected mean squares
Event	E_ℓ	L-1	$\sigma^2 + IJM\sigma_E^2$
Species	A_i	I-1	$\sigma^2 + L\sigma_C^2 + LM\sigma_B^2 + LJM\frac{\Sigma A_i^2}{I-1}$
Trees (species)	$B_{j(i)}$	$I(J$-1)	$\sigma^2 + L\sigma_C^2 + LM\sigma_B^2$
Points (trees species)	$C_{m(ij)}$	$IJ(M$-1)	$\sigma^2 + L\sigma_C^2$
Error	$RES_{ijm\ell}$	$(L$-1)$(IJM$-1)	σ^2

[a] L = number of storm events
I = number of tree species
J = number of trees within species
M = number of points sampled under each tree
ℓ = storm identification number, ℓ = 1,2,3
i = species identification number, i = 1,2,3
j = tree identification number, j = 1,2,3
m = point identification number, m = 1,2, . . . 15

$$\frac{\text{Expected Value of the Numerator}}{\text{Expected Value of the Denominator}} = \frac{\sigma^2 + L\sigma_C^2 + LM\sigma_B^2 + LJM(\Sigma A_i^2/(I-1))}{\sigma^2 + L\sigma_C^2 + LM\sigma_B^2} \quad (1)$$

where

L = number of storm events
J = number of trees within species
M = number of points sampled under each tree
$\Sigma A_i^2/(I - 1)$ = species variation
σ^2 = error variance
σ_B^2 = variance of trees within species
σ_C^2 = variance of points within trees and species

Equation 1 can be reduced to:

$$\frac{\text{Expected Value of the Numerator}}{\text{Expected Value of the Denominator}} = \frac{LJM(\Sigma A_i^2/(I-1))}{\sigma^2 + L\sigma_C^2 + LM\sigma_B^2} + 1 \quad (2)$$

from which the noncentrality parameter, λ, can be derived (Walpole and Myers 1978):

$$\lambda = \frac{LJM\Sigma A_i^2}{\sigma^2 + L\sigma_C^2 + LM\sigma_B^2} \quad (3)$$

A more practical and usable form of the noncentrality parameter is defined by ϕ^2 (Pearson and Hartley 1951), where:

$$\phi^2 = \lambda/((I - 1) + 1) \quad (4)$$

Substituting equation (3) into equation (4) and reducing the resulting equation yields:

$$\phi = \sqrt{\frac{\Sigma A_i^2/I}{\dfrac{\sigma^2}{LJM} + \dfrac{\sigma_C^2}{JM} + \dfrac{\sigma_B^2}{J}}} \quad (5)$$

Equation (5) is useful only if values for all variables other than J (number of trees per species) and M (number of points per tree) can first be estimated. Therefore, an analysis of variance for Table 1 was run for each chemical constituent, combining data for all three storms. From these analysis of variance results, estimates of $\Sigma A_i^2/(I - 1)$, σ^2, σ_B^2, and σ_C^2 were obtained (Table 2). The number of storms, L, and number of species, I, were both fixed at 3.

With all of this information, equation (5) was then used to determine the effects of the number of trees per species, J, and the number of points per tree, M, on sampling confidence for testing for species differences. For each nutrient, ϕ was calculated using many combinations of values for J and M, while holding the other variables constant.

TABLE 2. Estimates of the variance components for the new analysis of variance.

Constituent	$\Sigma A_i^2/(I\text{-}1)$	Variance component σ^2	σ_B^2	σ_C^2
NH_3-N	538.62	24.12	369.52	16.86
NO_3-N	445.83	91.91	995.85	132.88
Ca	2499.67	191.38	5686.48	420.89
Na	3.76	0.90	1.04	0.97
H	0.75	0.72	1.59	1.25
K	99101.91	2054.44	3402.56	2745.64

Once values for ϕ were calculated, the values of ϕ, υ_1 [numerator degrees of freedom of the F-ratio, equation (1)], and υ_2 [denominator degrees of freedom of the F-ratio, equation (1)] at $\alpha = 0.01$ were applied to Pearson and Hartley's (1951) Analysis of Variance Power Charts to determine power $(1 - \beta)$ values. Power is evaluated by the following probability function:

$$1 - \beta = P(F(\text{SPECIES}) > f_\alpha(\upsilon_1, \upsilon_2)|H_1) \tag{6}$$

where f_α is the critical F value at a desired confidence level given υ_1 and υ_2

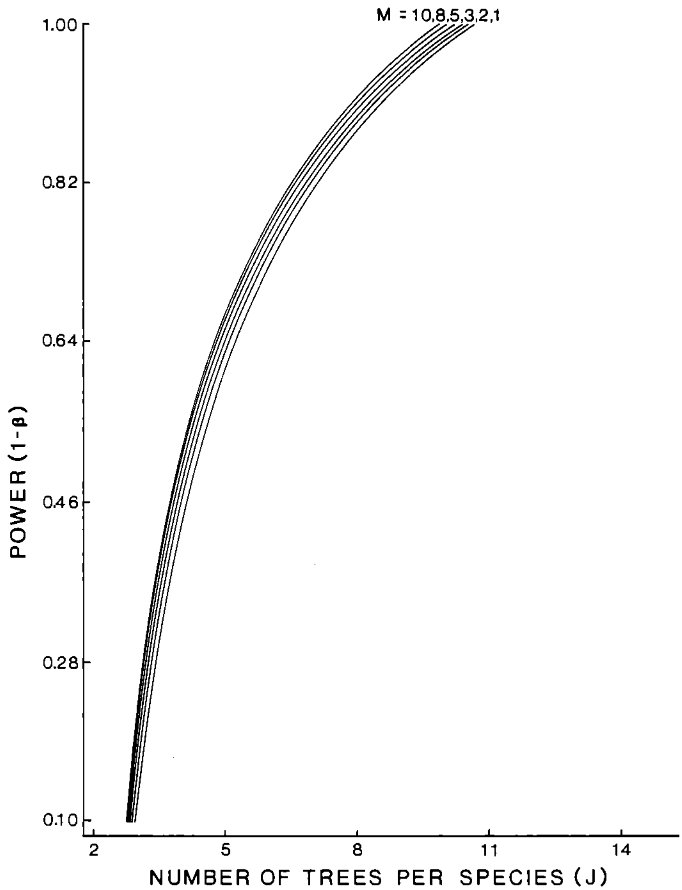

FIGURE 1. Power curves at $\alpha = 0.01$ for ammonia nitrogen sample-size determination. M is the number of points sampled per tree.

numerator and denominator degrees of freedom, respectively, and H_1 is the alternative hypothesis. In this case, the null hypothesis is that all the species' means are equal, and the alternative hypothesis is that at least one of the species' means is different than the other species' means. In other words, the power as defined in equation (6) is the probability of obtaining a significant test result when a difference in species' means actually exists (Walpole and Myers 1978). In this experiment, υ_1 was fixed at 2 since $\upsilon_1 = I - 1$, and the number of species, I, was fixed at 3. The degrees of freedom for the denominator, υ_2, varied depending upon the value of J since $\upsilon_2 = I(J - 1)$.

The power values $(1 - \beta)$ are plotted against the number of trees per species (J) at various levels of M (the number of points per tree) in Figures 1 through 5 for NH_3-N, NO_3-N, Ca, Na, and H loadings, respectively. A graph for potassium is not shown because the computed ϕ values were so small that they fell above the upper bounds of the Analysis of Variance Power Charts. This situation indicates that many samples are needed to obtain an adequately high power to detect potassium differences of the magnitude found in this study.

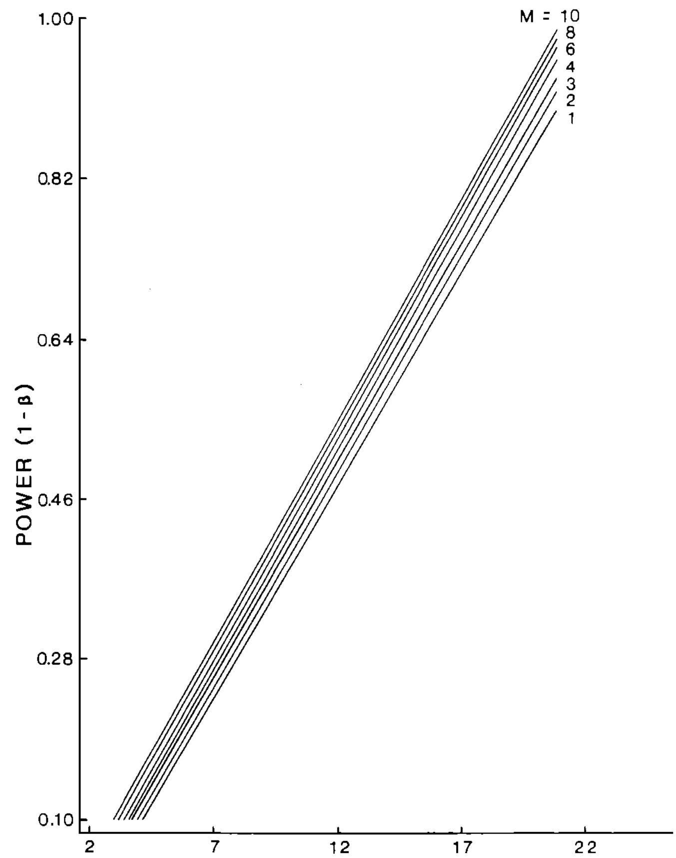

FIGURE 2. Power curves at $\alpha = 0.01$ for nitrate nitrogen sample-size determination. M is the number of points sampled per tree.

RESULTS AND DISCUSSION

Although determination of specific sample sizes was not the primary purpose of this paper, Figures 1 through 5 can be used as guidelines for determining the relative number of points per tree and/or trees per species required for determining species differences when analyzing throughfall chemistry. By choosing a desired power and keeping economic and other constraints in mind, an estimate of the number of samples can be obtained.

However, caution must be taken when using these graphs to define specific sample-size requirements. The probability of committing a type II error (β) is determined by α, the size of the population error variance, the sample size, and the magnitude of the difference between the true population parameter and the sample parameter under the null hypothesis. Since power ($1 - \beta$) depends upon β, power likewise depends on those same four factors. Only three storms and three species were used for calculation of the variables in equation (5). This small sample limits the accuracy of the variable estimates, which in turn affects ϕ. Also, the ΣA_i^2 values used in the calculation of ϕ are unique to this study. Specific sample sizes can only be determined from Figures 1 through 5 when looking for differences among species

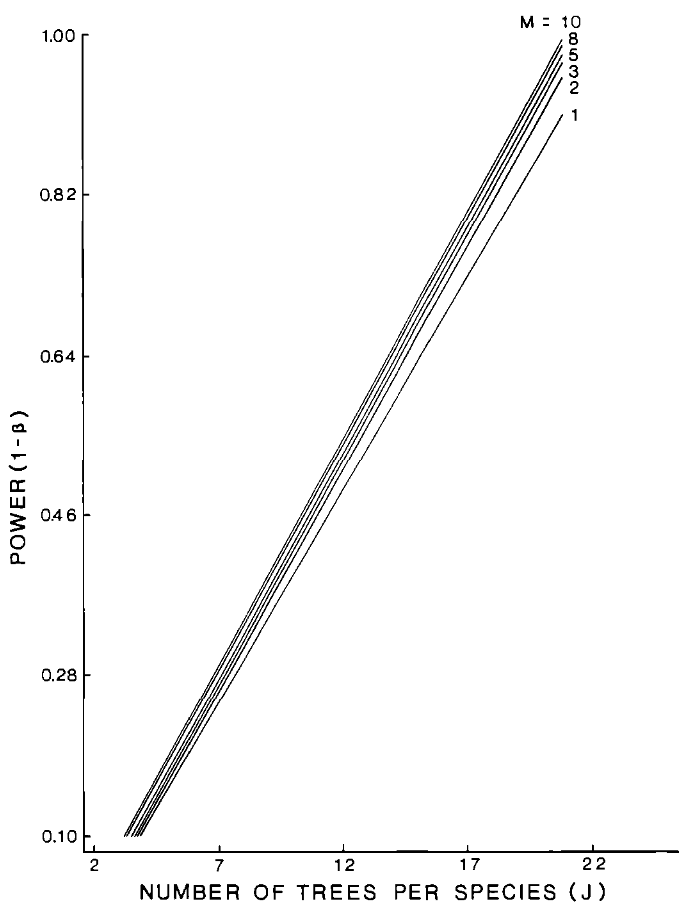

FIGURE 3. Power curves at $\alpha = 0.01$ for calcium sample-size determination. M is the number of points sampled per tree.

of the same magnitude as those determined in the present study. Smaller or larger differences will affect the power; the smaller the difference to be detected, the more observations needed to obtain a certain power at a particular significance level.

In general, any factors influencing throughfall will affect ϕ, which subsequently reflects upon the power. However, species, storm, and site variations will at worst probably cause changes only in the absolute power values. In addition, a different significance level will shift the curves up or down. These graphs are based on a significance level of 0.01.

The primary importance of the graphs presented here is to illustrate the relative changes in power that can be obtained by varying the number of trees per species and the number of points per tree sampled. Figures 1 through 5 demonstrate that the increases in statistical power (for testing for species' differences) resulting from increasing the number of trees within a species far exceed increases in power obtained from increasing the number of points sampled under any one tree. For example, the NH_3-N curves (Figure 1) show that increasing the number of trees per species from six to seven at one point per tree increases the power from approximately 0.74 to 0.81. In contrast, increasing the number of points per tree sampled from one

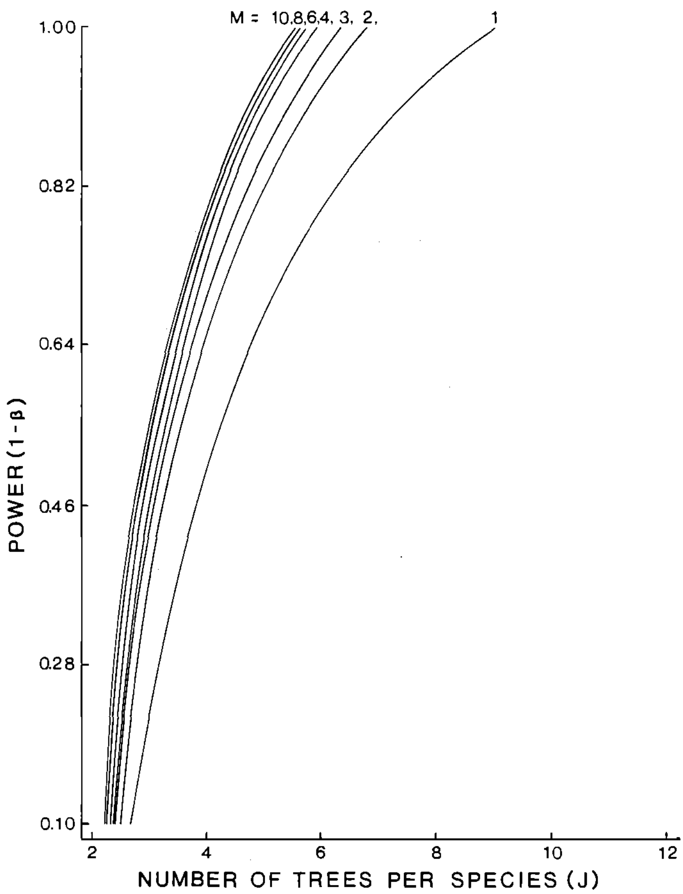

FIGURE 4. Power curves at $\alpha = 0.01$ for sodium sample-size determination. *M* is the number of points sampled per tree.

to two at six trees per species only increases the power from approximately 0.74 to 0.76. In fact, increasing the number of points per tree from one to ten at six trees per species only increases the power from about 0.74 to 0.79.

Note that to determine a sufficient sample size for the entire experiment, the same procedure for determining power must be carried out for all effects within the model. The largest number of samples determined for any of the effects should then be used for the whole experiment. The largest number of samples will meet the sample requirements for all other effects.

The same procedure outlined here could be used for any experiment. Frequently, power is ignored in statistical tests, and only confidence levels are given consideration. However, a test that has a high confidence level but low power is not desirable. More attention must be given to power. Power determinations may be time consuming and tedious for complex experimental designs, but results will be more accurate and informative.

CONCLUSIONS

The complexities involved in throughfall sampling are not always immediately apparent. Many factors must be considered, including both the

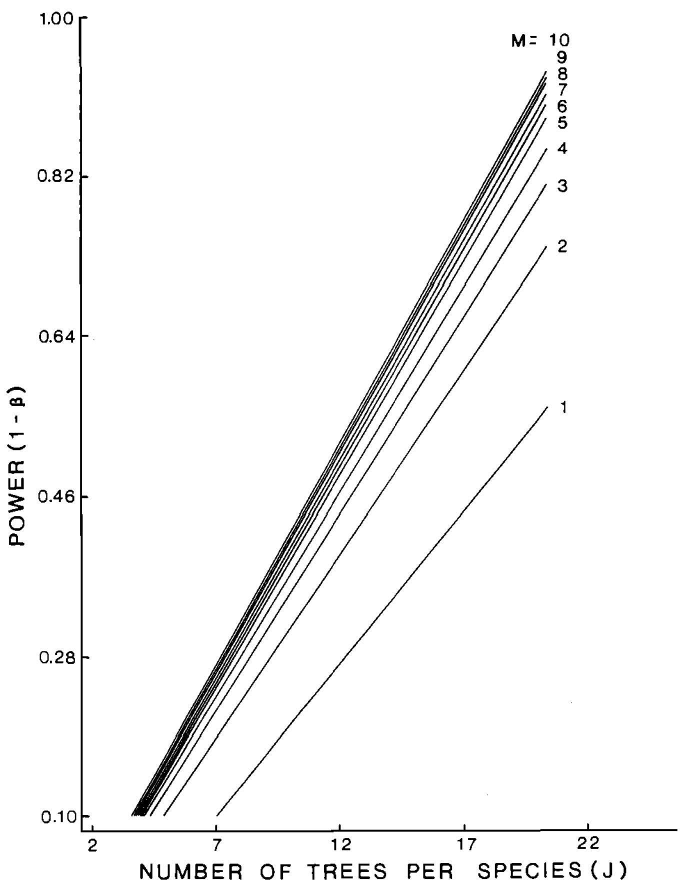

FIGURE 5. Power curves at $\alpha = 0.01$ for hydrogen sample-size determination. M is the number of points sampled per tree.

number of points per tree and the number of trees per species required to collect a representative sample. Based on the model and variance estimates used herein, the gain in statistical power obtained from increasing the number of trees per species by one at any number of points per tree was greater than any gain in power obtained from increasing the number of points per tree by one at any number of trees per species. This result was shown for NH_3-N, NO_3-N, Ca, Na, and H for the test for species differences. The reader should be aware that future studies may not behave like this one. Differences in experimental design, improvements in the accuracy of variance estimates, etc. may result in situations where increasing the number of points per tree could improve power considerably.

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