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Extractive contents and fungal degradation of branches from well-pruned and poorly-pruned mature Virginia pine[1])

A Note

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Introduction

Natural pruning ability of forest tree species has generally been considered a function of stand density (SMITH, 1962). Canopy development of full-stocked stands gradually decreases levels of solar energy needed to support the photosynthetic activity of lower crown branches causing their gradual suppression and ultimate death. Dead and dying branches are subsequently attacked by fungal agents weakening their attachment to the tree bole. The structurally-weakened branches are then sloughed from the stem by gravitational and wind forces.

The retention time of branches after death may be influenced by branch diameter or by qualitative and/or quantitative antifungal extractive elements within the branch tissue. Fungal agents probably require longer time intervals to structurally weaken large diameter branches than small branches. The fungitoxic capacity of extractive elements or specific chemical compounds contained in branchwood extractives may be additional factors responsible for natural pruning characteristics. If chemical properties of branch extractives and branch size are indeed important factors related to natural pruning ability, elucidation of the genetic control of these characters would be important for genetic improvement of selected forest tree species. Investigation of pruning in terms of retained dead branch susceptibility to wood decaying fungi, although implied in the literature (CHANG, 1962; SLOCUM and MILLER, 1953), has not been previously considered.

Pinus virginiana (MILL.) normally exhibits poor natural pruning characteristics even in densely stocked stands. Retention of dead branches for extended periods has been attributed to the large amounts of durable branch heartwood (SLOCUM and MILLER, 1953). Presumably, the chemical components in the wood complemented by branch size provides resistance to fungal degration.

Genetic variation for natural pruning in P. virginiana was found on the Lee Experimental Forest[3]) by BAILEY et al. (1974). Adjacent well-pruned and poorly-pruned mature P. virginiana were observed growing (under similar environmental conditions) at various locations on the forest.

The primary objectives of this study were to compare rates of fungal degradation of dead retained branches from the two selected populations and to compare the percentage and phenolic composition of ethanol-benzene extractives in dead branches of the two select populations.

Literature Review

Natural Wood Durability

Wood durability of living and harvested timber is of considerable interest to plant pathologists. Research has focused primarily on host-saprophyte- and -parasite relationships involved in natural fungal inoculation and rates of cellulolytic degradation (HART and HILLIS, 1972; HANOVER and HOFF, 1966; JORCENSEN, 1961; LI et al., 1969; LOWMAN, 1970 a; LOWMAN, 1970 b; RUDMAN, 1963; and SHRIMPTON and WHITNEY, 1968). Due to the unique chemical composition of pine extractives (MIROV, 1961) a substantial amount of research has been attempted to determine the importance of these extractives as factors responsible for natural resistance to wood decay (JORGENSEN, 1961; LOWMAN, 1970 b; SHRIMPTON and WHITNEY, 1968; and THIELGES, 1968).

Much of the research attempting to determine the fungitoxic effects of extractives employ in vitro procedures. RUDMAN (1962, 1963) believes erroneous results may be obtained from such techniques and the use of natural substrates in their original form provides results more comparable to natural conditions. Extrapolation of in *vitro* techniques may be complicated by antagonistic or synergistic effects of host extractive compounds (LI et al., 1969; LI et al., 1972). Despite the complexities encountered when assessing the inhibitory effects of extractives on fungal growth by in vitro techniques, such methods do provide valuable information concerning the possible mechanisms responsible for maintaining wood integrity.

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Materials and Methods

Branch Extractives

Six pairs of naturally well-pruned and poorly-pruned trees (tree numbers 1, 2; 19, 20; 37, 38; 43, 44; 45, 46; 47, 48) located throughout the Lee Experimental Forest were chosen for experimentation. The well-pruned and poorly-pruned trees were represented by odd and even numbers, respectively, and were a random subsample of 48 trees originally selected on the Lee Forest (Bailey *et al.*, 1974).

Ten dead branches were randomly selected and removed from each tree at varying intervals along the stem between 2.2M and 12.2M. Three sample discs, 3.2 mm thick, were cut from each branch of each tree adjacent to branch attachment to the bole. The thirty discs representing each tree were separated into three replicates. Each replicate consisted of ten discs representing the original branches removed from each tree. Discs were macerated to a uniform size (2 mm) in a Wiley Mill. Five one-gram samples were taken from each replicate. The samples were oven-dried at 80° C for 72 hours and oven-dry weights recorded. Resin was extracted by means of a Soxhlet apparatus as described by Barger and Folliot (1971).

After extraction, wood meal samples were ovendried under the same conditions as before and re-weighed to determine the percent (on a weight/weight basis) resin content. The extractives were recovered and adjusted to equal concentrations (g extractives/ml) by a rotary-flash evaporator. The adjusted aliquots were stored in the dark at 4° C under nitrogen.

Thin-Layer Chromatography

Stored resin extracts were chromatographed in an attempt to separate and compare qualitative branch phenolic variation between the poorly-and-well-pruned groups. A solvent system composed of benzene and glacial acetic acid 9 : 1 (v/v) was used. Silica-gel, thin-layer plates 5 × 20 cm were used to observe one-way separation of branch phenols. The chromatographic tanks were equilibrated for a two-hour period before prepared plates were emersed in the solvent to minimize "edge effects". Phenolic separation was observed by irradiating the silica-gel plates under ultraviolet light (315—400 nm). Relative mobility values (Rm) were calculated for individual phenols by using a standard phenol which migrated the furthest from the point of resin application. This phenol was found to be present in all samples by co-chromatography of sample mixtures. Three phenol samples from each selected tree were run to enable calculations of phenolic Rm variability.

Fungal Isolation

From the original parent branches used in the extraction procedure (excluding parent trees 1, 2 and 19, 20 because of both space limitations in incubation chambers and lack of sample materials), two replicate discs 6.4 mm in width, were cut from each branch. Branches were surface sterilized by soaking in ten percent Chlorox bleach solution for approximately three minutes before the discs were cut. Discs were cut using saw blades sterilized with 95% ethanol. Discs were placed in petri plates containing glucose yeast extract agar (GYEA) which consisted of 5 g D-glucose, 1 g yeast extract, 20 g agar per liter distilled water. The medium was amended with 200 μ g/ml chloremphenicol to exclude bacterial growth. Plates were incubated at 27° C. Fungi appearing on the wood discs after a period of ten days were recorded according to branch number and parent tree. Generic classification of fungi was accomplished using gross morphological characteristics; sporulating forms were microscopically observed and keyed to species when possible. A total of eleven fungi were observed; eight of the most common fungi were isolated and stored in an incubator at 5° C. The eight fungi were saved for use in the decay analysis of branch discs from well- and poorly-pruned trees. Only four fungi were identified; *Pestalotia* sp., *Trichoderma viride, Pullularia pullulans* and *Hypoxylon* sp.; *Pestalotia* sp. and *Pullularia pullulans* have been reported to cause considerable amounts of wood decay, particularly under moist conditions (Duncan, 1960). *T. viride* has been reported by Nelson (1972) to be responsible for cellulose degradation. Hulme and Shields (1970) have observed *T. viride* to inhibit certain wood-decaying fungi. They theorized that *T. viride* retards growth of other fungi by depleting wood of its non-structural carbohydrates such as polysaccharrides and starch. These compounds are thought to be the initial substrates for various wood saprophytes.

The four remaining non-sporulating, unidentified fungi were included as inoculants in an attempt to simulate natural conditions.

Fungal Decay Analysis

Two wood discs 1.75 mm in width were removed from each branch of the sample trees (tree numbers 37, 38; 43, 44; 45, 46; 47, 48) at the point of branch attachment to the stem. Each individual tree was therefore represented by twenty branch discs. Discs were ovendried for 72 hours at 105° C, weighed and placed in distilled water to establish a moisture regime favorable for fungal growth. After an equilibration period of 48 hours, discs were removed from the beakers and placed in individually prepared petri plates (9 × 15 cm). Moistened filter paper was placed on the bottom and lid of the culture plates to maintain a high moisture regime. Discs were supported on bent (V-shaped) glass tubing to prevent physical contact with the filter paper. Culture plates containing wood disc samples were autoclaved for twenty minutes prior to inoculation.

All discs were individually infected with the eight previously-isolated fungi. Plugs (3 mm in diameter) were removed from the periphery of vigorously growing cultures and placed mycelium side down on the wood discs. Petri plates containing the inoculated discs were placed on trays and enclosed completely in polyethylene bags to limit moisture depletion during the incubation period. Discs were incubated at 27° C. The decay analysis was terminated after seventy days. Agar plugs were removed from the wood discs after which the discs were ovendried and reweighed.

Two control groups, representing the well- and poorly-pruned populations, were incorporated into the experiment. Each control group was represented by twelve wood discs randomly selected from each population. The control discs were subjected to the same treatments described above, but were not inoculated.

Results

Resin Extractive Study

Individual mean branch extractive content for each tree is tabulated in *Figure 1.* In each instance, with the exception of trees 45 and 46, branches of the poorly-pruned

parent trees had higher extractive contents than the corresponding branches of the well-pruned parent trees.

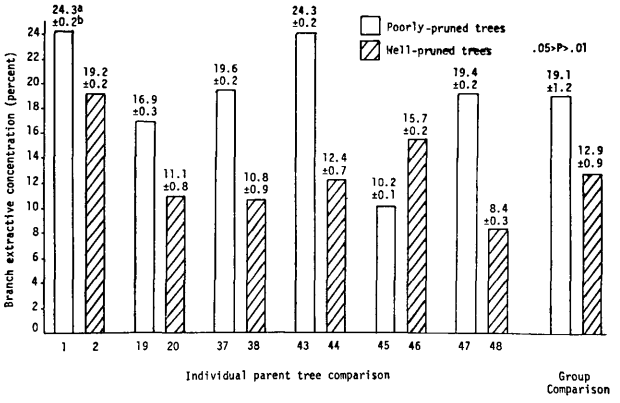

Figure 1. — Percent branch extractive concentration in well-pruned and poorly-pruned *P. virginiana* trees and combined group comparison. — a = mean; b = SE.

Percent extractive variables were pooled into poorly- and well-pruned groups and analyzed by a two level pure model II nested analysis of variance (Sokal and Rolf, 1969). A significant difference ($.05 > P > .01$) was observed between the poorly- and well-pruned parent branch groups.

Chromatographic analysis of phenolic composition of extractives from the well-pruned and poorly-pruned tree branches revealed thirteen phenols. It was not the purpose of this experiment to isolate and identify specific phenols in the branch extractives of the selected trees. No meaningful qualitative phenolic dissimilarities between the poorly- or well-pruned branch extractives were observed although variation among trees was evident.

Decay Analysis

Figure 2 illustrates results of decay analysis of branch wood discs from the well- and poorly-pruned parent trees. Percent weight loss per unit disc area was used to analyze the degradative effects of fungi on the wood discs. A Wilcoxon two-sample test, as described in Sokal and Rolf (1969), was used to test the hypothesis that the two inoculated populations did not differ in relative weight loss (expressed as: [(100) (initial wt. — final wt.)/ initial wt.]/ cm^2.) The null hypothesis was rejected at the 0.01 significance level, indicating a significantly greater weight loss occurred in discs from the well-pruned trees than occured in discs from the poorly-pruned trees *(Figure 2).*

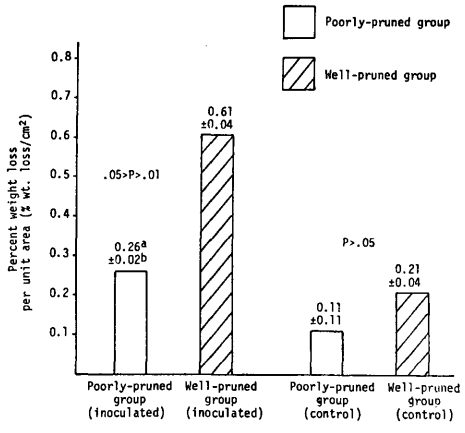

Figure 2. — Comparison of percent weight loss per unit area of inoculated and control branch discs from poorly-pruned and well-pruned *P. virginiana* trees. — a = mean; b = SE.

Results of analysis of the control groups indicated no significant difference in weight loss among the two groups. A small amount of abrasion did apparently occur during the preparation and weighing of the discs causing a small weight loss difference between the control groups.

Discussion

The primary objective of this study was to assess natural pruning potential of selected poorly- and well-pruned *P. virginiana* trees by monitoring fungal degradation of wood discs from retained dead branches. Fungal degradation, measured in terms of wood disc weight loss, was employed as an indicator of relative loss of strength of retained branches at the point of attachment to the bole.

The results of this study indicate a significantly greater wood disc weight loss in inoculated branches from the well-pruned group. The well-pruned group also possessed significantly smaller amounts of ethanol-benzene extractives in their dead retained branches. Branch size of this group was also significantly smaller than that of the poorly-pruned group suggesting a relationship between branch size and heartwood content or formation. The results support the suspected relationships of natural pruning potential of the two selected populations (Bailey *et al.*, 1974). The concept of general extractive concentrations inhibiting fungal growth is further supported by the failure to find meaningful qualitative phenolic differences between the branches of the well-pruned and poorly-pruned groups.

Several other mechanisms have been suggested as responsible for fungal inhibition other than those of chemical toxicity of extractive compounds. Rudman (1962) considered the function of extractive elements in a moisture limiting capacity producing suboptimum conditions for fungal growth. This mechanisms may have application to the results observed in this study; profuse mycelial mats were observed on both well-pruned and poorly-pruned wood discs at the termination of the isolation and inoculation procedures. The greater percent weight loss per unit area of well-pruned discs suggests that internal as well as external wood decay may have been occurring. The poorly-pruned wood disc group may not have had internal mycelial growth because of high extractive concentrations.

If branch extractives contribute to inhibition of fungal degradation, as the results indicate, genetic investigation to elucidate the genetic control of these extractives is warranted. Although no genetic estimates of *P. virginiana* branch extractive concentrations have been cited in the literature, Thor (1964) has reported large variation of bole extractive concentrations among *P. virginiana* stands located in Kentucky and Tennessee (0.014—0.052 g/cm^2). A moderately high parent-progeny correlation ($r = 0.38$) of ethanol-benzene extractives for *P. elliottii* has also been reported (Franklin and Squillace, 1973). The genetic relationship of branch extractives and branch size and angle should also be investigated. Consideration should be given to the genetic relationships which may exist between branch and bole extractives. Since extractives appear to be instrumental in suppressing wood decaying fungi, selecting trees with low branch extractive concentrations may result in breeding bole susceptibility to decay fungi. A determination of phenotypic and genotypic correlations of these traits will be necessary prior to initiating a breeding program.

Abstract

Naturally well-pruned and poorly-pruned *P. virginiana* trees were selected. Branches from the select trees were used for determination of dead branch extractive content, phenol analysis and decay analysis. Although no phenolic differences were observed between well-pruned and poorly-pruned groups, extractive content and decay rates differed significantly. Branches from poorly-pruned trees contained 7.2% more extractives than branches from well-pruned trees. Decay rates (% weight loss/unit area) of branches from well-pruned trees were 35% geater than those from poorly-pruned trees. Results indicate that branch extractive content is one factor contributing to the natural pruning ability of *P. virginiana*.

Key words: *Pinus virginiana* (Mill), pruning, fungi. branch retention, extractives.

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Photosyonthetic CO_2-Uptake and the Distribution of Photosynthate as Related to Growth of Larch and Sycamore Progenies

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Introduction

The growth of agricultural crop plants has been greatly improved through the efforts of plant breeders. It has become obvious that selective breeding can also improve the growth of trees to meet better the requirements of society (e. g. Zobel 1971), but there is a basic difference between agricultural crops and trees in the speed with which improvement can be attained. One of the most needed innovations in tree breeding is a technique for predicting the breeding value of species, provenances, strains, or families based on juvenile evaluation of their progeny. Even an imperfect correlation might increase genetic gain if it shortened the selection cycle sufficiently (Nanson 1970).

Our objective was to develop techniques useful for predicting which tree progeny or species was superior in growth. The present investigation represents a step in this direction, not a definitive result. Predictions of relative growth were made with a simulation model using as input: rates of photosynthetic CO_2-uptake, respiratory CO_2-emission, and parameters reflecting the way in which photosynthate is allocated for growth of leaves and other organs (see Ledig 1974, 1969 for development of the model).

Prior to the present report, it had not been demonstrated that genetic variation in growth could be explained or predicted by growth models even in the short term. CO_2-exchange rates alone had failed to explain genetic variation in growth at least as often as they had aided such explanations, resulting in hesitance to pursue such techniques in breeding. Nevertheless, on balance the evidence suggested that the methods described below would be useful in predicting genetic differences in growth and might be developed as a potentially powerful tool for tree breeders.

Materials

Representatives of two deciduous genera, sycamore (*Platanus occidentalis* L.) and larch (*Larix* sp. Mill.), were