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United States
Department of
Agriculture

Forest Service

**Southern Forest
Experiment Station**

New Orleans,
Louisiana

General Technical Report
SO-101
March 1994

Symposium on Current Research in the Chemical Sciences

Third Annual Southern Station Chemical Sciences Meeting

Third Annual Southern Station
Chemical Sciences Meeting

SYMPOSIUM ON CURRENT RESEARCH IN THE CHEMICAL SCIENCES

Alexandria, Louisiana
February 7-8, 1990

Edited by Timothy G. Rials

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Preface

The original charter for this annual meeting of chemical sciences personnel called for an informal atmosphere for the discussion of common concerns and needs. The years have seen the definition of our "common concern" evolve into a sharing of our efforts in applying the science of chemistry to the resolution of problems faced by our forest resource. I believe this expanded scope is significant because, as we pursue our special interests (physiology, ecology, utilization), it is easy to forget that the objective — to enhance the security of the concept of multiple-use forestry — remains constant. This affords several advantages, including an enhanced awareness of the breadth of chemical sciences research that is being conducted, as well as an increased familiarity with project resources and individual skills that are available across the Southern Station's laboratories. An additional benefit to be gained through this approach can be found in the diverse backgrounds, philosophies, and perceptions that are collectively available to address research problems. This meeting provides an opportunity to generate new approaches and new perspectives far removed from those applied previously.

This report contains proceedings of the Third Annual Southern Station Chemical Sciences Meeting. The collection of papers is divided into three sections addressing forest biology, forest protection, and forest utilization. It effectively covers *the life cycle of the forest* with topics ranging from seed coat physiology to the development of new adhesives for wood composites. This report also illustrates the far-reaching role of chemistry through the discussion of subjects as fundamental as polyflavanoid stereochemistry and as applied as nitrogen fixation in agroforestry systems. In short, this proceeding defines the wide range of research problems whose solution lies somewhere in the realm of chemistry.

I would like to express my gratitude to all of the participants in this symposium, and especially to the authors who have contributed to this effort. I hope that this has proved to be as beneficial an exercise to all of you as it has to me. Also, there are a number of individuals whose contributions made the meeting a success, and they deserve a special note of thanks. They include Mr. Raymond Paul and Mr. Mike Roessler who handled dinner accommodations, Miss Debbie Wolfe who compiled this document, and Mrs. Becky Rials who did whatever else was required. Finally, let me express my appreciation to R.W. Hemingway whose assistance in every aspect of organizing this meeting was invaluable. Thank you all!

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Integration of Ion-Selective Electrodes into a Laboratory Analysis System

Roger L. Baker

INTRODUCTION

Ion-selective electrodes (ISE's) allow the specific and quantitative determination of substances, ranging from simple inorganic ions through amino acids to complex organic molecules. About 30 anions can be specifically and directly detected with these electrodes, but in addition, other ions can be determined indirectly through chemical complexation, precipitation, or biochemical reactions that change the activity of the indicated ion in the solution equilibrium (Cammann 1979).

ISE's appear to be an alternative to expensive equipment often used for ion analysis. The wide range of applications, low material requirements, and simplicity of the analytical procedures have brought ISE's into active use in analytical chemistry for many disciplines. When compared to the initial and per sample costs of other methods, the ISE's appear to be an answer for the small, budget conscious lab. Time-consuming sample preparation including filtrations, distillations, and extractions can often be avoided through the use of ISE's. Methods of analysis by ISE's are usually nondestructive and adaptable to very small sample volumes. Analysis can be made of highly colored or viscous samples without difficulty.

The ISE's are not without problems though. Cammann (1979) estimated that most problems relate back to a lack of practical know-how on the part of the user and to difficulties with the reference electrode. In addition, direct, interference-free measurements of desired ions can be rendered difficult due to interferences from other ions.

Ion-selective electrodes sense the activity rather than the concentration of ions in solution. The ISE's are electrochemical half-cells in which a potential difference that is dependent on the concentration or activity of a particular ion in solution arises across the electrode/electrolyte interface (Cammann 1979). These interfaces are regions of space that separate phases in such a way that material transport between the outer, contacting phase is modified or inhibited compared to transport that would occur when phases are in direct contact (Cammann 1979, Lakshminarayanaiah 1976).

Membranes are generally liquids or solids and are usually thick enough that they possess an inside region and two outer-boundary-defining surfaces (or interfaces) that separate the membrane from the exterior phase. Each interface is a hypothetical surface that separates the physical-chemical properties of the membrane from the outer phases where another set of chemical and physical properties exists. Location of the interfaces is not absolute because important properties such as charge density and potential distribution vary continually from one phase to another. Membranes for ISE's are immiscible or at least partly so with respect to the bathing solutions or solid contacts. Hydrophobic organic liquids and low water-solubility organic solids constitute the main materials of membrane construction.

Membrane ISE's are commonly classified according to the type of membrane. The first ISE was actually a glass pH electrode that measured the activity of the H^+ ion. A complete description of the different types of membrane electrodes that have been developed is available in Bailey (1976), Cammann (1979), and Ma and Hassan (1982). The types of ion selective electrodes that are readily available commercially are classified as: (1) liquid membrane electrodes, (2) sealed or solid-state electrodes, and (3) combination electrodes.

Liquid membrane electrodes have a screw-on sensing module that contains an inert membrane through which a layer of liquid ion exchanger establishes contact with the sample. The exchanger is a water-soluble salt of an organophosphoric acid and has high specificity. A gelled salt bridge solution connects the liquid membrane with an internal Ag-AgC1 element. This type is represented by the calcium and divalent ion electrodes.

The solid-state or sealed electrode is the most basic type. A sensing membrane is permanently mounted in the tip, and an ion potential develops across that membrane. Solid-state electrodes require a reference electrode for use.

Combination electrodes incorporate both a solid ion-sensing element and a reference electrode in a single stem. These electrodes can be used with very small samples. Gas-sensing combination electrodes have been developed that use a hydrophobic gas-permeable membrane to separate sample solution from the electrode internal solution. Dissolved gas in the sample solution diffuses through the membrane until the partial pressure of the gas is the same on both sides of the membrane.

CALIBRATION

Regardless of the evaluation method being employed, the electrode should periodically be tested by means of a calibration curve. Normal procedures start with a 0.1 M solution of the indicated ion and progressively dilute until no further dilution effect can be detected by the detecting instrument. The calibration curve is obtained by plotting the measured electro-motive force (EMF) values on a linear ordinate scale against concentrations on a logarithmic abscissa scale. The lower detection limit is defined as the measured ion concentration at which the measured signal is exactly twice as large as the background noise (Bailey 1976, Cammann 1979).

Standards should bracket the concentration ranges as closely as possible. The analytical measurement can never be obtained more accurately than the calibration standards are known or defined. Care is required, especially when

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working with standard solutions less than 10^{-5} M, since highly diluted, unbuffered solutions are unstable due to irreversible absorption processes on the container walls. Therefore, such solutions should always be freshly prepared.

MEASUREMENT

The most important and widely used techniques for measuring ion activity are as follows: (1) direct indication/concentration, (2) standard addition/subtraction, (3) Gran's plot, (4) potentiometric titration. These are noted in increasing order of accuracy and precision and time required per analysis.

1. Direct indication/concentration. This is the most straightforward technique and is the one of choice whenever possible. It is a rapid technique enabling measurements to be completed in just 2 to 3 minutes and is the most familiar and simple technique for workers. Typical accuracy of measurement is approximately ± 2 percent (Bailey 1976, Cammann 1979).

2. Standard addition/subtraction. In the standard addition method, the ISE is immersed in the sample and the equilibrium cell potential is recorded. A known volume of a standard solution is then added, and the new equilibrium cell potential, the change in potential, is measured. The initial concentration of the sample may then be calculated from the change in potential (Bailey 1976, Orion 1986). The known addition is useful for measuring dilute samples, checking results of direct calibration, or measuring total concentration of an ion in the presence of an excess complexing agent.

3. Gran's plot. This method locates the equivalence points in titrations based on linear titration curves. The volume of titrant consumed is plotted on Gran's plot paper against the concentration of the ion to be determined, and a straight line is obtained. As the volume of titrant added increases, the concentration of the ion decreases linearly to approach zero at the equivalence of the end point (Bailey 1976, Ma and Hassan 1982, Orion 1982).

4. Potentiometric titration. In potentiometric titration, the sample is titrated with suitable titrant, and the decrease or increase in titrant activity is followed with an ISE. The end point of the titration is assumed to be at the inflection point of the curve. Titrimetry is seldom the preferred manual technique. Usually, when accuracy and precision are important, the analysis may be done more rapidly and the end point located more precisely by Gran's plot method, if available. The titration does enable a large number of ions not directly sensed by ISE to be measured (Bailey 1976, Ma and Hassan 1982).

EXPERIENCE

The author's approach to the use of ISE's was one of optimism and of naiveté. As Durst (1978) states "... it is usually not until one is actually using these sensors that the problems and pitfalls become apparent, and may result in serious errors and considerable aggravation to the unwary. In many cases, the problem results because the analyst has not familiarized himself with the particular idiosyncrasies of his electrode system...often, the users approach the application of ion-selective electrodes with great anticipation, assuming that since they have used the pH electrode without difficulties, the analogous ion-selective electrodes will be equally well behaved. This naive approach often leads to considerable disappointment because the potential sources of trouble are almost as varied as the sensors and the samples...common causes of difficulty can be identified: electrode interferences and/or "fouling" of the sensors, sample matrix effects, reference electrode instability, and improper calibration of the measurement system."

The study with ISE's began with a need for determining sulfate, orthophosphate, nitrate, and ammonia concentrations from samples of rainfall, throughfall, and litter leachate. Several companies have ISE systems available (Cammann 1979). A high-quality pH/ISE meter, a printer, a reference electrode, and the fluoride, lead, and ammonia ISE's were purchased. Total investment (suggested retail price) would be about $3,700. Additional investments were made in pipettes, tips, and disposable polypropylene beakers.

Through the use of Gran's plot titrations, sulfate ion concentration can be obtained using the lead electrode (titrate with lead perchlorate), and phosphate ion concentration can be obtained using the fluoride electrode (uses lanthanum nitrate and sodium fluoride). Satisfactory results were not obtained for either ion. In retrospect, the results fit our overall knowledge concerning sample analysis with ISE's. Most samples contained less than 0.1 ppm total phosphorous and 1.0 ppm total sulfur, which approach the lower detection limits of phosphate and sulfate. At times, it was difficult to even plot the blank and standard. Currently, the phosphorous and sulfate analyses are run by a local soil-testing lab, but the ISE phosphate and sulfate analyses will be attempted using soil samples with higher levels of each ion in the samples. These analyses will be approached with a better understanding of ISE's and the perils of their analysis.

No major problems have slowed ammonia and nitrate measurement. The ammonia electrode with the nitrate test kit is used for nitrate measurement, and is less subject to interference from other ions than the nitrate electrode. The nitrate test kit requires a different filling solution for the ammonia electrode. There are two ammonia electrodes, one electrode specifically for ammonia determination and one for nitrate determination.

An oxygen electrode is currently used to measure dissolved oxygen in surface and ground water samples. This electrode has been trouble-free, and it can be used in the

determination of biochemical oxygen demand from future samples.

Many minor problems have been faced during the 2 years of sample measurement (or attempted measurement) but have been overcome. Good lab procedures and recordkeeping must accompany the sample measurement. All chemicals should be dated when received and again when opened. The shelf life of unopened standard solutions is about 3 years, but once opened, their shelf life would be reduced to 1 year. One standard solution could not be calibrated correctly after repeated tries because of the age of the solution.

Equipment is available to automate the ISE sample measurement, although the investment for that type of system runs from $15,000 to $20,000. This cost is approaching the investment required for other methods of sample measurement. However, that would be a top-quality ISE system, and measurement of additional ions could be added to the system for a relatively small investment.

Future plans call for ISE measurement of chloride and aluminum (fluoride electrode). These measurements will be approached with a better understanding of the operations of ISE's and an awareness of possible troubles.

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Comparing Elemental Compositions from Seed Leachates and Seed Coat Surfaces of *Pinus taeda*

J. A. Vozzo

INTRODUCTION

Leachate analyses of seeds imbibed in deionized water have shown a relationship between seed health and electrical conductance (Bonner 1988, Bonner and Vozzo 1987). The poorer the seed quality of southern pines, the more electrical conductance their leachate allows in deionized water. Although the quantitative conductance may be measured in μmhos by any of several techniques, there is no clear qualitative analyses of the leachate. Additionally, Vozzo and Song (in press) have proposed that a structural failure or trauma of cell membranes in stressed seeds allows more than usual active and passive translocation, thereby contributing to additional leachates and a higher conductance from poor seeds in water. Data here present a view of associating electrical conductance from leachate with leachate analyses by atomic absorption and seed coat elemental analyses using energy dispersive spectroscopy (EDS).

MATERIALS AND METHODS

Seed samples of loblolly pine were collected and selected for a representative of a poor seed source and a good seed source. Individual seeds were randomly selected from each source to provide replications of each analytical technique.

Using these sources as examples of the relationship between conductance and germination, seeds of both sources were analyzed for a possible source of conductance by qualitative and quantitative analyses of elements present in their leachate and seed coat surfaces.

Conductivity readings were collected using the YSI32 conductivity meter. Seeds were placed in 100 ml of deionized water for 24 hours of imbibition, then strained from their leachate. Electrical conductivity for leachates and water controls was recorded in units of μmhos/gram of seed. The YSI32 conductivity meter has a built-in temperature compensation. Bonner (1988) describes the use of both multiprobe and single-probe conductivity meters for seed leachate analyses.

Qualitative and quantitative leachate analyses were modified from atomic absorption and spectrophotometric methods developed for liquid samples by Switzer and others (1988) and Worsham (1989, personal communications). Nine grams of each seed sample were shaken with 25 ml of double deionized water overnight at room temperature. Extractions were filtered using a vacuum flask and 0.22 μfilters. A Perkin Elmer 5000 was used for atomic absorption observations and the Milton-Roy 1001+ provided spectrophotometer data. Data reflect the average from three replications of 9-gram samples of the poor seed source and the good seed source.

For EDS, 10 seeds of each source were observed at 3 random spots on the seed coat of each seed sampled. Seeds were attached to opaque carbon planchets with small amounts of epoxy and placed in an AMR 1000A scanning electron microscope (SEM) equipped with a Kevex 5100 energy dispersive x-ray spectrometer. Seeds were coated with carbon using a Polaron Sputter Coater equipped with a carbon evaporation unit to minimize specimen charging. Data were collected with the following conditions being held constant: magnification, duration of probe (200 sec), accelerating potential (30 KV), tilt (45°), consider lens setting (spot size 3), and working distance (12 mm). Spectral data were transmitted to a computerized smoothing routine followed by background subtraction. X-ray counts were recorded for each integrated elemental peak and total counts for the spectrum, after background subtraction. The elements detected were expressed as a percentage of total counts after background subtraction.

RESULTS

The good seed source (LOB 55) germinated 80 percent and averaged 10.5 μmhos/gram seed weight, whereas the poor seed source (LOB 14) germinated 1 percent and averaged 60.2 μmhos/gram. Germination was tested for 28 days under standard germination conditions.

Leachate from each seed source in deionized water was analyzed by atomic absorption/spectrophotometry. Nine elements were detected in each of both seed sources. In ascending atomic number, sodium (Na), magnesium (Mg), phosphorous (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) were found in significantly different amounts for each source's leachate (table 1).

Seed coat surface elemental composition was determined by EDS. In this analysis, eight elements were detected on the seed surface as follows in order of ascending atomic number: aluminum (Al), silicon (Si), P, sulfur (S), chlorine (Cl), K, Ca, and Fe. Of these eight seed coat surface elements detected, only Ca is in significantly different amounts between both seed sources, with the low conductance source having twice as much Ca present as the high conductance source (table 2).

Among the total 13 elements found, only P, K, Ca, and Fe were commonly detected in both the leachate and on the seed coat surface with only Ca different in both analyses (fig. 1). As seen in tables 1 and 2 and figure 1, the primary source of ion contamination in the deionized water is from the leachate coming out of the seed. This discounts the seed coat contamination as the probable cause for increased conductance in bad seeds.

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Table 1.--*Elemental analysis of seed leachate from good seed source and poor seed source* Pinus taeda *seeds, expressed in ppm*[*]

	Na	Mg	P	K	Ca	Mn	Fe	Cu	Zn
Good seed source	6.32 A	1.04 A	5.33 A	55.29 A	0.30 A	0.20 A	0.61 A	5.33 A	0.09 A
Poor seed source	9.38 B	24.06 B	26.03 B	439.33 B	4.61 B	3.00 B	2.47 B	0.14 B	0.55 B

[*]Means not sharing letter in common differ significantly at 0.01 probability level by Duncan's Multiple Range Test.

Table 2.--*Elemental analysis of seed surface from good seed source and poor seed source* Pinus taeda *seeds, expressed in share of total counts of spectral energies generated after background subtraction*[*]

	Al	Si	P	S	Cl	K	Ca	Fe
Good seed source	1040 A	16449 A	109 A	658 A	263 A	1777 A	2719 A	266 A
Poor seed source	1121 A	15036 A	217 A	552 A	281 A	1855 A	1300 B	631 A

[*]Means not sharing letter in common differ significantly at 0.01 probability level by Duncan's Multiple Range Test.

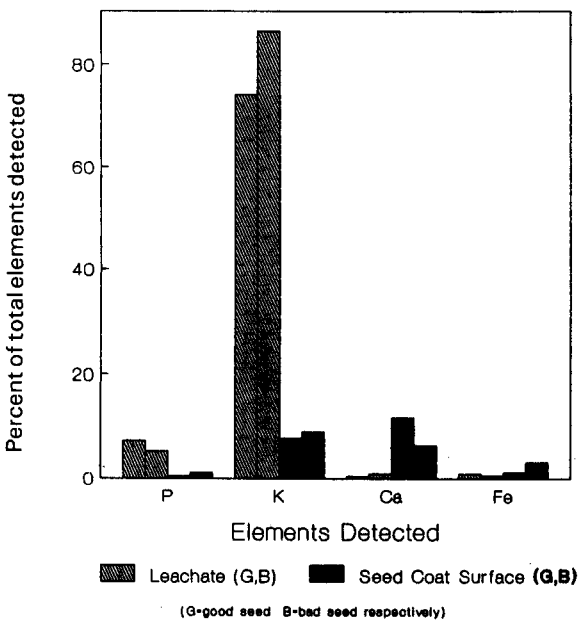

Figure 1.--*Percent of each element detected in both leachate and seed coat surface analysis.*

DISCUSSION

As the conductance of electrical current is dependent upon ionic presence of contaminants in deionized water, the higher conductance of LOB 14 than LOB 55 reflects a greater amount of ionic loss from poorly germinating seeds than from better ones.

Ca ions alone do not explain the increased conductance by poorly germinating seeds as the better seed source shows twice as much Ca loss as the poorer seed source. The increased Ca coming from the seed coat surface alone does not affect germination (table 2). However, the disproportionately greater amounts of leached ions from the poor seed source found by atomic absorption do readily indicate the source of conductance as coming from inside the seeds. The one exception is Cu (table 1).

There is a high level of Al from seed coat surfaces detected by EDS. Al x rays may be impinging from the stage assembly of the instrument itself. Even though the seeds were mounted on carbon planchets, there is a possibility of beam interaction with the stage. This allows a possible source of Al x-ray excitation directly from the stage or scattering from the curved seed coat surface.

The greater amounts of ions detected in the leachate than on the seed coat surface, however, indicated that seed coat contamination is not as major a contributor to conductance by poor seeds as is the inherent potential of the seed itself to leak ions when damaged.

Two important studies are now underway to supplement this hypothesis. First, additional seed rinsing prior to conductivity tests are in progress by Bonner (1989, personal communications). Secondly, high-voltage electron microscopy studies by the author indicate cell wall trauma associated with high conductance seeds. A cell wall trauma would allow more ions to leach from the poorer seeds than an intact cell wall allows from the low conductance seeds.

ACKNOWLEDGMENTS

The author wishes to acknowledge the following individuals for expertise in their respective interests: F. T. Bonner, single-probe electrical conductance; H. M. Worsham, atomic absorption and spectro-photometry; Ray Scheetz, EDS instrumentation.

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Application of the Incubation, Drying, and Separation Method to Pinus roxburghii Seeds

R. V. Singh and J. A. Vozzo

INTRODUCTION

The cultivation of coniferous plants in containers is increasing in temperate zones of the world. Economically, it is desirable to make use of every seed as well as each container; i.e., to sow only one seed per container. This depends on high seed quality if the percentage of empty containers is to be kept low. Each container requires the same time for seedling production and management, whether a plant develops in it or not. Improvement of the seed germination percentage can decrease the number of empty containers. Therefore, it is highly desirable to remove the dead seeds from a lot before precision sowing can be done.

Simancik (1965) studied the differences in the rates of water uptake of empty, dead, and viable seeds for grading the seed quality of several coniferous species. Seeds placed in water sank to the bottom at different treatments depending on their viability. However, a separation of viable and nonviable seeds cannot be achieved by this method.

Simak (1970) has shown that *Pinus silvestris* seeds, after being soaked in water for a few hours and subsequently dried, will lose their water at different rates depending on their viability. The water is lost faster from dead seeds than from viable seeds. This led to a new technique developed for separating filled-live from filled-dead lodgepole pine seeds that relies on incubation-drying-separation (IDS) regimes (Simak 1983, 1984). The IDS-method is based on the previously described principle that viable seeds after incubation (I) and subsequent drying (D) lose absorbed water at a much slower rate than dead seeds. This causes differences in weight and densities between live and dead seed and makes it possible to use separation methods (S) based on physical principles such as flotation and gravity separation.

The objective of this study was to determine the incubation, drying time, and separation gradients (solvent, solute, and their specific gravities) for separation of filled-live from filled-dead *Pinus roxburghii* seeds.

MATERIALS AND METHODS

The seeds used in this experiment were purchased from Mistletoe Sales - S&S Seeds, 5690 Casifas Pass Road, Carpinteria, CA.

R.V. Singh is visiting scientist from G.B. Pant University, Ranichauri, India; J.A. Vozzo is research plant physiologist at Forest Sciences Laboratory, U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Starkville, MS 39759.

Sucrose was dissolved in distilled water to make solutions with specific gravities of 1.02, 1.04, 1.06, 1.08, 1.10, 1.12, and 1.14. Distilled water was used as a control. Specific gravity was determined by using standard hydrometers of laboratory grade.

Separation of the seeds was carried out by the IDS method as follows:

I (Incubation): Four hundred seeds were initially radiographed to determine empty and full seeds. Then, all seeds were placed in an incubator between two moistened blotter paper sheets and kept in this condition in a germinator for 72 hours at 15 °C, 100 percent RH, and 1,000 lux illumination.

D (Drying): After incubation, the seeds were surface dried and spread out in a layer on blotter paper for 2-, 4-, 8-, and 16-hour drying.

S (Separation): After drying, the seeds were placed in eight specific gravity solutions of sucrose and distilled water. After 5 minutes, the buoyant seeds on the surface were removed and counted. They represented the dead seed fraction. The sunken seeds on the bottom represented the viable seed fraction. They were separated and counted. All the sinking and floating seeds were placed in the germinator.

Germination was carried out at 20 °C/30 °C and observed for germination after 7, 14, 21, and 28 days. Seedlings having root lengths three times the length of seed were considered as germinated. At the end of the germination test, all ungerminated seeds were inspected by a cutting test.

In this IDS study, the seeds were designated empty or full. The following categories were established:

Good ungerminated seeds	= full, live
Abnormal germinated seeds	= full, live
Rotten seeds	= full, dead
Empty seeds	= empty, dead.

In addition to the initial radiography and IDS-method to determine full and empty seeds as well as live and dead seeds, other seed sample history was analyzed: weight per 1,000 seeds, percentage of moisture loss in fresh seeds at 2-hour intervals for 16 hours, and percentage of moisture loss in germination twice in a week up to 28 days.

RESULTS

Average weight of *Pinus roxburghii* seeds was 110.4 g/1000 seeds. The range of moisture content loss in dry seeds was 7.6 percent to 9.3 percent at 2-hour intervals over a 16-hour period (fig. 1). In dry seeds and 16-hour imbibed seeds, moisture content loss was 8.5 percent and 32.7 percent respectively. During the germination trials, moisture content losses varied from 37.9 percent to 73.7 percent while germination increased 8 percent to 43 percent (fig. 2).

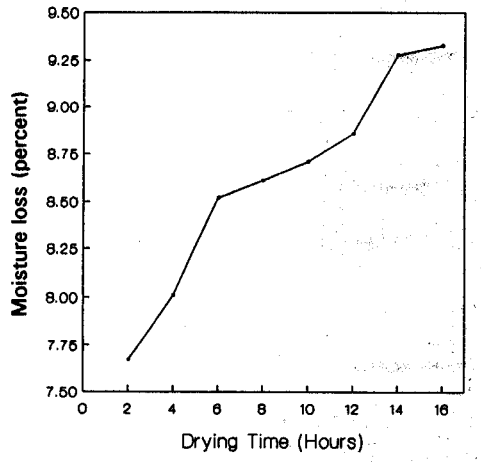

Figure 1.--*Percentage moisture loss in dry seed from 2 hours to 16 hours drying.*

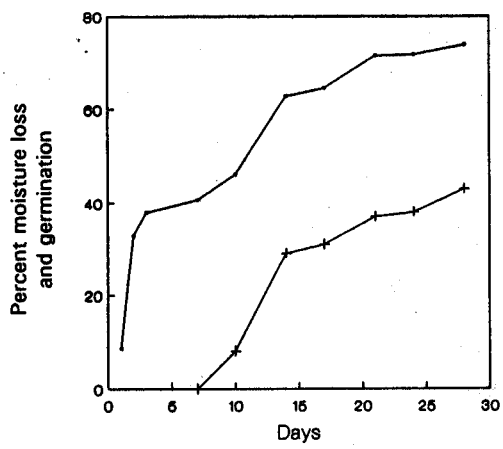

Figure 2.--*Percentage moisture loss in dry, 16 hours imbibed and germinated seeds, and germination percentage of seeds.*

In radiography tests, an average 11 percent empty seeds were observed in the 4-hour drying trial followed by 8 percent, 5 percent, and 4 percent in 2-hour, 8-hour, and 16-hour drying trials. No more than 5 percent damaged seeds were recorded in 16-hour drying trial (fig. 3).

The object of these preliminary trials was to determine the combination of drying time and sucrose gradient that gave separation of filled-live seeds comparable to those from germination trials. *Pinus roxburghii* had 73 percent normal germination, so the goal was to find the combination of drying time and gradient to separate 27 percent of the seeds. According to the IDS-principle, the drying created a difference in the water content and consequently in the density between dead and viable seeds.

Two combinations (i.e. 4-hour drying, separated by 1.04 specific gravity sucrose solution, and 8-hour drying, separated by 1.06 specific gravity sucrose solution) separated filled-live from filled-dead *Pinus roxburghii* seeds (table 1, figs. 4, 5).

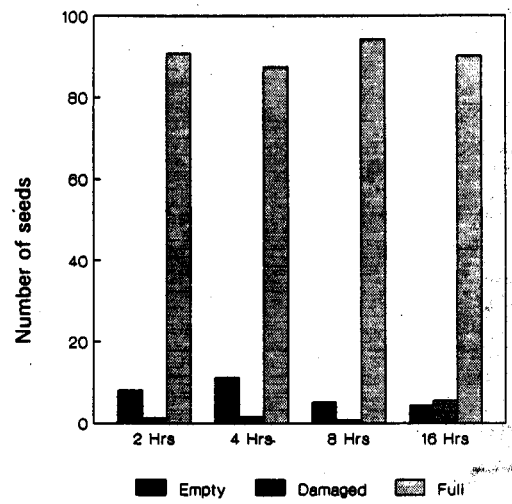

Figure 3.--*Characteristics of seed lot x-radiography.*

There are two reasons for a certain mixture of viable and dead seeds in the floating and sinking fractions, respectively. First, some of the germinable seeds have a low viability, and their ability to retain water is low when they are dry. Consequently, some of the seeds are removed together with the dead seeds during the separation step. The number of viable seeds in the floating fraction can be adjusted to some extent by changing the length of the drying time after incubation. Second, mechanically and insect-damaged seeds easily absorb water and sink to the bottom together with the viable seeds during the separation step.

The radiographs clearly show that the full and empty seeds can be separated by the IDS-method with good accuracy. The ungerminated seeds in sink- and float-fractions at the end of the germinated test were analyzed by a cutting test showing a few ungerminated seeds in float-fraction were viable but failed to germinate.

The results of IDS-preliminary trials indicate that there is a need for further research to determine the optimum combinations of drying times and separation gradients for separation of filled-live from filled-dead *Pinus roxburghii* seeds.

Table 1.--*Germination (percent) of IDS-treated Pinus roxburghii seeds*

Drying time	Specific gravity of solution	Fraction	Percent in seed fraction	Germination percent
--	1.0, D.W.	control	100	73
4-hours	1.04, sucrose	sink	72	94
		float	28	50
8-hours	1.06, sucrose	sink	74	86
		float	26	15

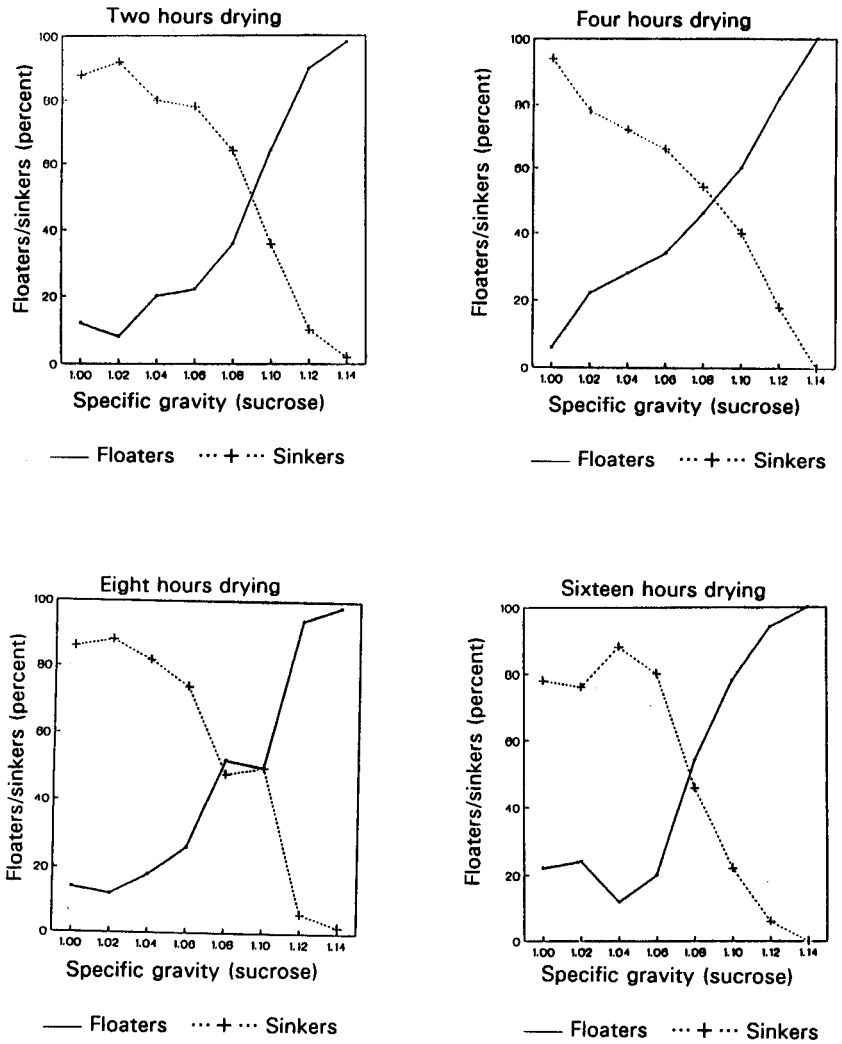

Figure 4.--*Floater and sinker seed (percent) in different specific gravity solutions.*

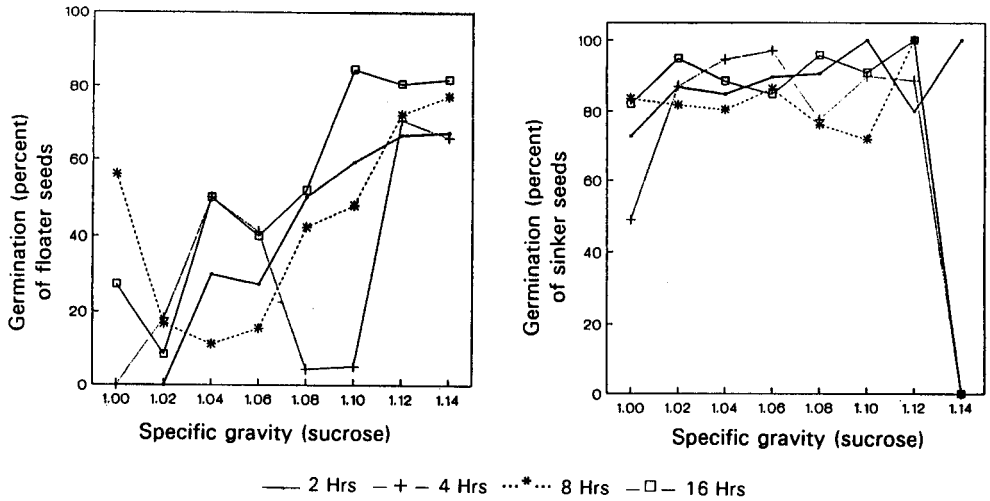

Figure 5.--*Germination (percent) of floater and sinker seed in different specific gravity solutions at 2-16 hours drying.*

CONCLUSION

Separation of *Pinus roxburghii* seeds by the IDS-method gave positive results. Seeds with 73 percent normal germination were clearly enhanced after IDS-treatment. Removing 28 percent of mostly dead seeds after a 4-hour drying period at room temperature by 1.04 specific gravity sucrose solution increased the germination rate to 94 percent.

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Comparative Seed Coat Anatomy of Dormant, Stratified, and Germinated *Pinus koraiensis* Seeds

Zhuahua Ning, J. A. Vozzo, and M. V. Bilan

INTRODUCTION

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is a five-needle, mountain tree species with natural distribution in northeastern China, southeastern Siberia, South Korea, and central Honshu in Japan. This pine begins to produce cones at the age of 80, but good seed production can rarely be expected until the tree is 120 years old. Large crops usually occur at 2- to 3-year intervals.

The long period of seed dormancy and the low percentage of seed germination are the major problems in establishing Korean pine. Some researchers reported that seed dormancy depends on the abscissic acid balance in the seed (Chen and Wang 1987). Others investigated the relations between seed coat and dormancy. Wang and others (1986) suggested the Korean pine seed dormancy has little relation with mechanical resistance or gas impermeability of hard seed coat and perhaps it depends on hormonal balance in the seeds. But Zhang and others (1981) reported that its hard testa constituted the main barrier to respiratory gas exchange, especially the penetration of oxygen into the seed. They propose that this might be a factor in dormancy control, whereas the inner coat appeared to affect seed germination ability in a manner unrelated to gas exchange.

Stratification remains the best pretreatment for overcoming dormancy and hastening seed germination. Asakawa (1955, 1956 a,b, 1961, and 1964) reported that rudimentary and dormant embryos enlarged during stratification. He also found that during pretreatments, glucose and stachyose appeared, whereas sucrose and raffinose decreased. He further mentioned that the embryo enlargement may also be explained by an increase in the ability to absorb water after pretreatment. Chen and Wang (1987) stated that during stratification the abscissic acid level decreased in the outer seed coat and endosperm but increased in the inner seed coat and embryo.

In this paper, scanning electron microscopy (SEM) photomicrographs show comparative seed coat anatomy of dormant, stratified, and germinated Korean pine seeds.

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MATERIALS AND METHODS

Seeds were collected from a natural Korean pine forest on Lesser Xinggan Mountain, Heiliongjiang, China, in 1983 and stored at 4 °C. Before stratification, seeds were soaked in running water at 15 °C for 1.5 days, then stratified at 4 °C. After stratification for 4 months, seeds were placed in a germinator at alternating 20 °C to 30 °C with 12 hours of photoperiod until the radicle emerged 7 mm in length. Seed coat samples were collected separately from dormant, stratified, and germinated seeds. Three-mm squares were cut from the seed coats. Squares were fixed in 4 percent glutaraldehyde buffered with 0.07M cacodylate at pH 7.2 for 1 hour. After being rinsed three times in the buffer, squares were dehydrated in a graded ethanol series and dried in a DCP-1 Critical Point Dryer, then coated in a Hummer Sputter Coater. All observations were made with a Hitachi S-405A scanning electron microscope.

RESULTS

The mature Korean pine seed is 16-17mm long, obovoid in shape, and has a yellowish brown color. The seed coat has three distinct layers (fig. 1): a thin sclerified outer layer formed by 3-4 layers of macrosclereids, a thick sclerified middle layer formed by 10-15 layers of osteosclereids, and a thin, papery membranous layer (fig. 2).

The seed surface (outer layer of the seed coat) appears to be layered also, with a wax and lipid coating layer on top (fig. 3), a large, narrow macrosclereid layer on the bottom, and a netlike matrix in between (fig. 4). Surface texture of dormant seeds was different from that of stratified and germinated seeds by having obvious wax and lipid deposition (fig. 3), whereas the amount of wax and lipid on the seed surface decreased during stratification and germination (fig. 5, 6). The wax and lipid top layer and the netlike matrix almost disappeared during seed germination (fig. 6). There are definite pores on the surface of the stratified and germinated seeds (fig. 5, 6). The pores vertically run through outer and middle layers of seed coat. The outline of each cell on the surface of stratified and germinated seeds can be seen more clearly than on dormant seeds (fig. 4, 5, 6). The intercellular spaces are larger on the surface of stratified and germinated seeds than on dormant seeds (fig. 4, 5, 6), perhaps due to decreased wax on their seed surfaces.

The middle layer of seed coat consists of 10-15 layers of osteosclereids (fig. 1, 7), which are typically bone shaped, with secondary walls and very small cell lumen. The secondary wall shows prominent simple pits (fig. 7, 8). The middle layer of the seed coat of stratified and germinated seeds is less dense than that of dormant seeds.

The inner layers of the seed coat of dormant, stratified, and germinated seeds are similar. This layer is derived from the inner, fleshy layer of the integument. During seed maturation, the fleshy layer of the integument is crushed and

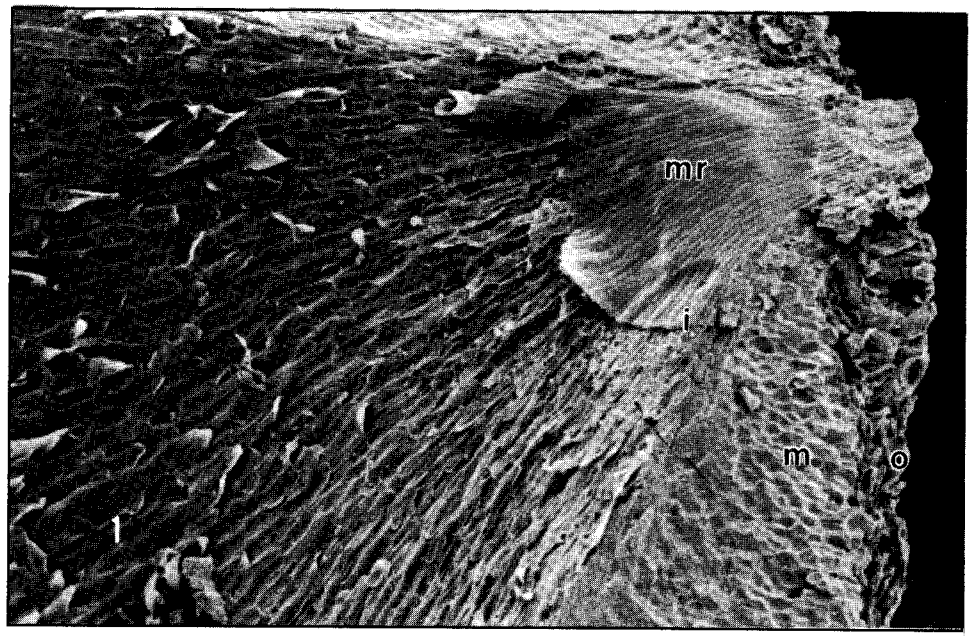

Figure 1.--*Seed coat of Pinus koraiensis, x 50; o = outer layer of seed coat; m = middle layer of seed coat; i = inner layer of seed coat; mr = micropylar region.*

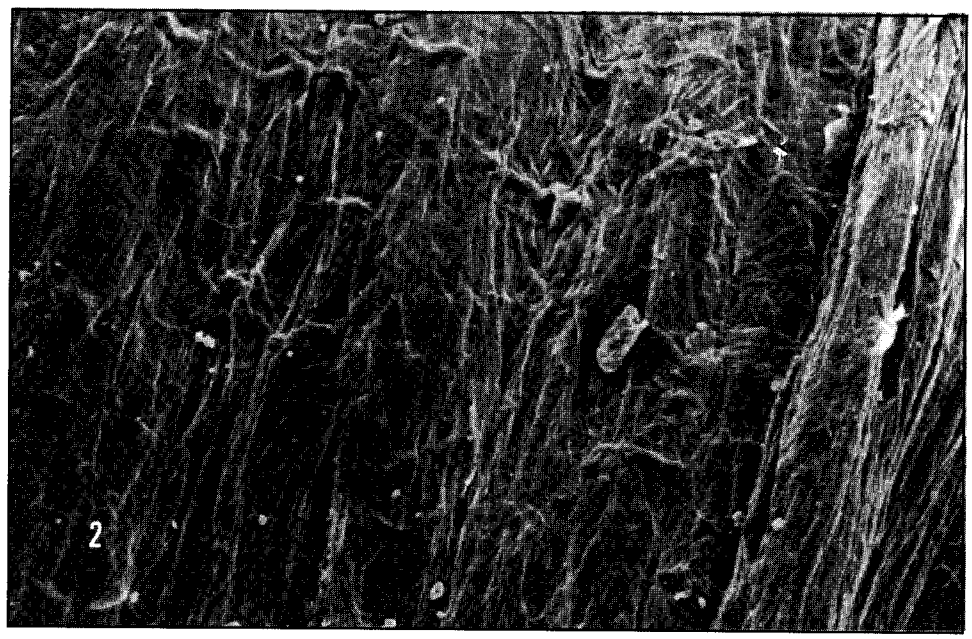

Figure 2.--*Inner layer of seed coat, x 250.*

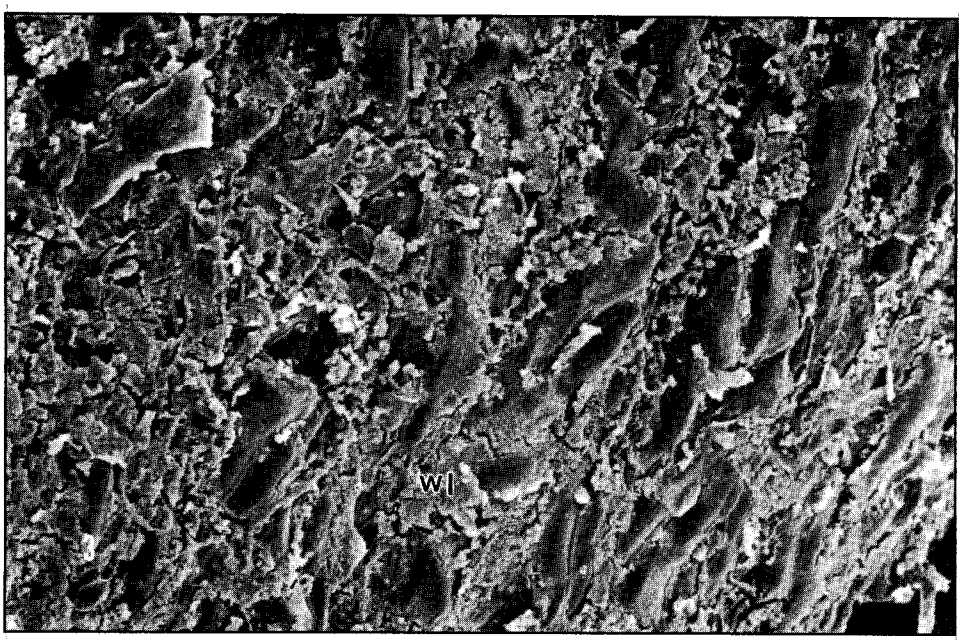

Figure 3.--*Surface of dormant seed, x 150; wl = wax and lipid deposits.*

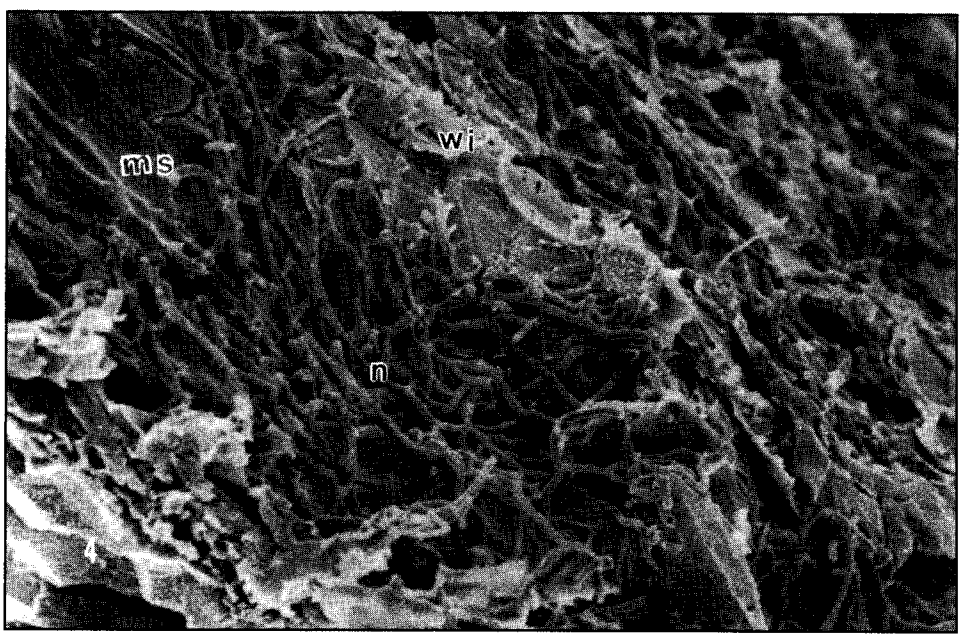

Figure 4.--*Surface of dormant seed, x 250; n = net layer on the seed coat surface; MS = macrosclereid layer on the seed coat surface.*

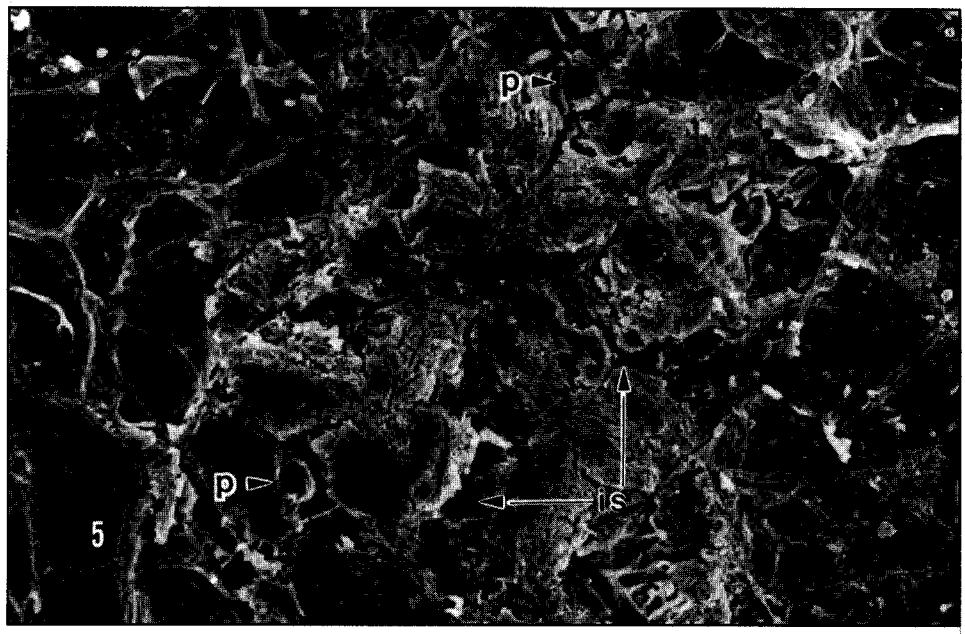

Figure 5.--*Surface of stratified seed, x 500; p = pore; is = intercellular space.*

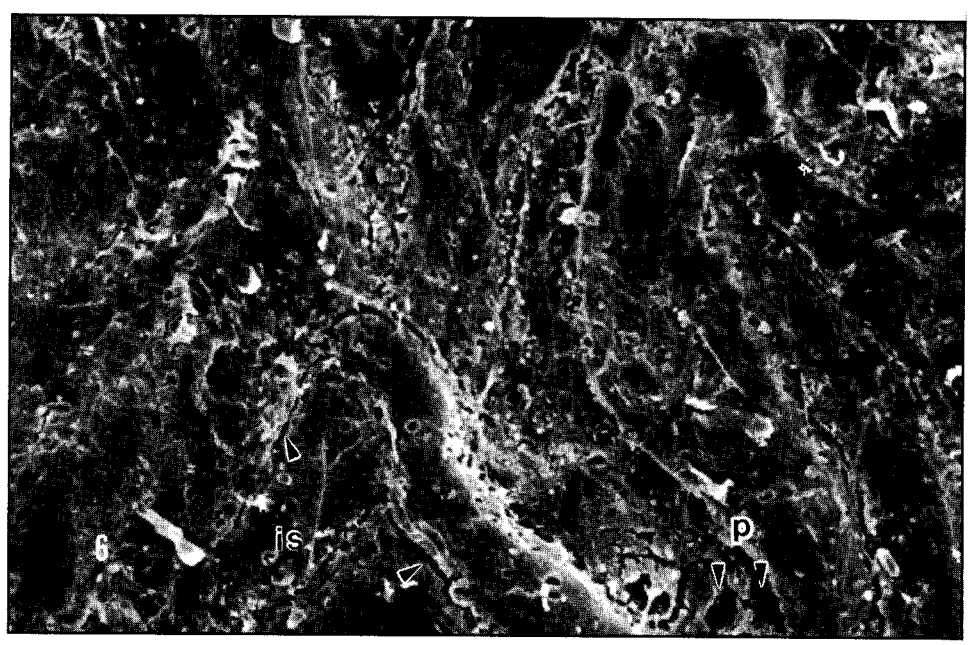

Figure 6.--*Surface of germinated seed, x 300.*

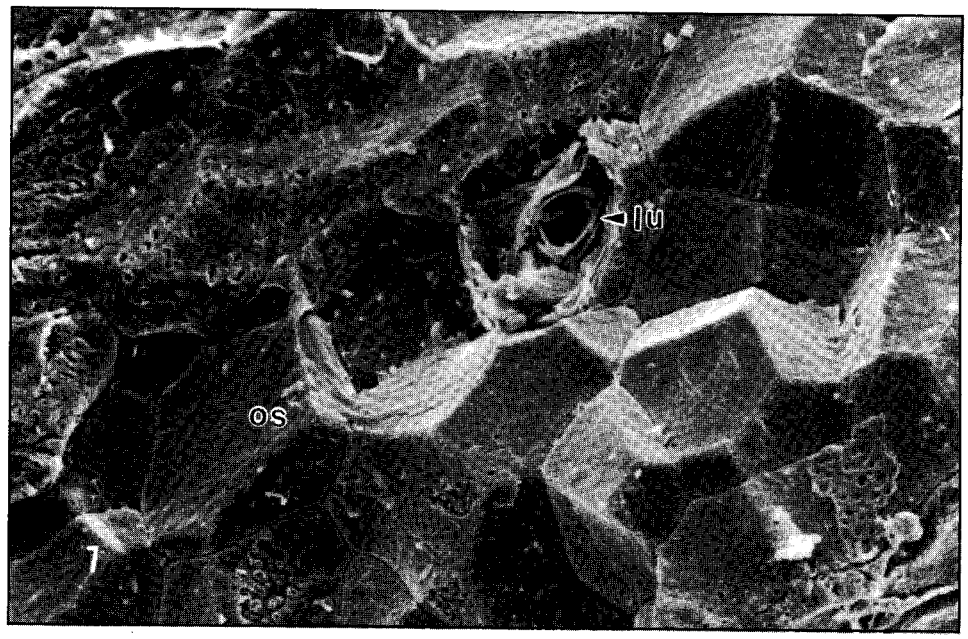

Figure 7.--*Middle layer of seed coat of stratified seed, x 500; os = osteosclereid; lu = cell lumen.*

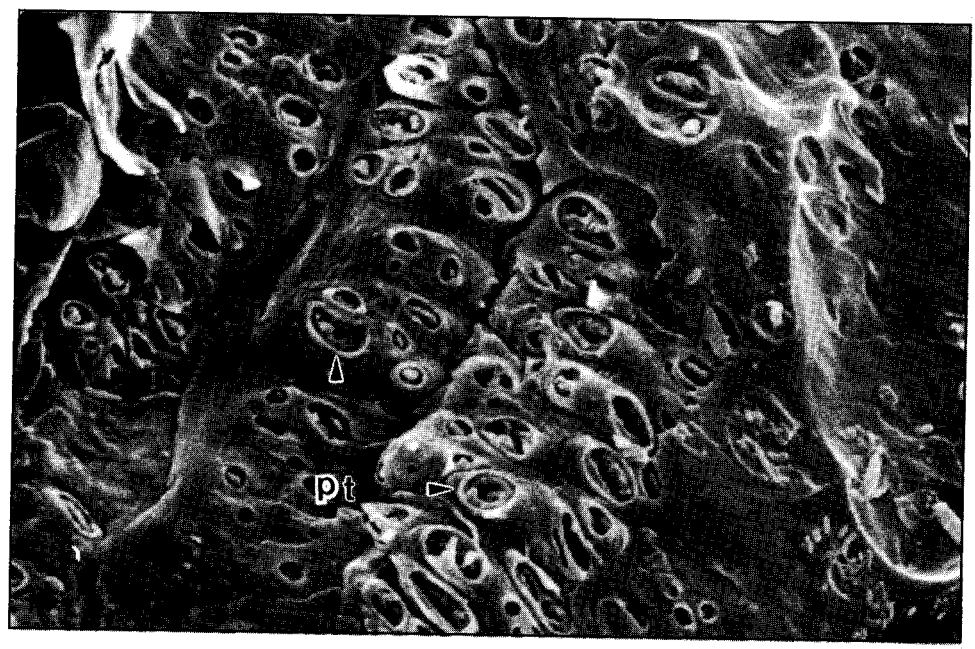

Figure 8.--*Middle layer of seed coat of germinated seed, x 1500; pt = pit.*

pressed into a papery membrane that lines the seed coat and wraps around the gametophyte and remnants of the nucellus.

DISCUSSION

The stratification treatment influenced both rate and germination. Seeds stratified for 90 days germinated 18.51 percent after 30 days, whereas seeds stratified for 120 days germinated 38.95 percent after 30 days (fig. 9). Unstratified seeds barely germinated. Germination tests verified that the longer the stratification period, the higher the germination percentage. Some researchers suggested that Korean pine seeds should be stratified for at least 6 months (Xu 1978).

Entry of water into the seed is greatly influenced by the nature of the seed coat. Heavy deposits of wax and lipid on the surface of dormant Korean pine seeds may repel moisture because of their hydrophobic characteristic. These deposits also plug the pores and intercellular spaces on the seed coat. After stratification, the amount of deposits decreased. The decrease may be partially due to soaking the seeds in running water for 1.5 days before stratification and partially due to the stratification process itself. During soaking, the running water forced seeds to rub against each other, affecting the integrity of the wax and lipid layer on the surface of the seed coat. During stratification, the deposits were further decomposed.

Like *Pinus elliottii* and *P. taeda*, Korean pine seed surface has a netlike matrix. Vozzo (in press) reported that this may suggest a hyaline layer of fungal presence, indicating a symbiotic relation between seeds and fungi. Stratification may provide a moist environment for fungal growth on the seed coat surface, and fungi may serve as a scarification process that would help water and gas exchange to satisfy prerequisite germination conditions. Fungi may also decompose the deposits of wax and lipid on the seed coat surface during stratification.

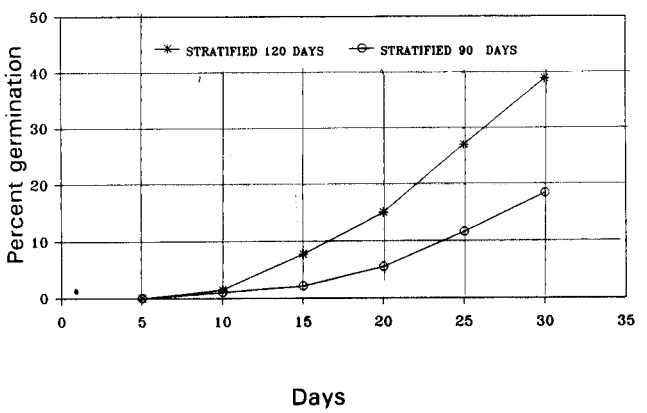

Figure 9.--*Germination of Korean pine seeds after treatment.*

During stratification, the seed coat density decreased. The cleft of the seed coat in the micropylar region loosened to allow increased imbibition and also lessened the mechanical resistance of the seed coat to later radicle emergence during germination.

Pores running through the seed coat may offer channels for water and gas exchange. The pits on the surface may also function as translocation channels, since the thick secondary wall layers in the pit region were completely interrupted.

Further studies will emphasize elemental composition of the seed coat; ultrastructural changes; and translocation patterns of carbohydrates, lipids, and proteins of dormant, stratified, and germinated Korean pine seeds.

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Subterranean Clover: Nutrients For Pines

Henry A. Pearson, Michael L. Elliott-Smith,
Alton Martin, Jr., and Virgil C. Baldwin

INTRODUCTION

Forest soils in the South are generally low in available nitrogen. Therefore, legumes and other nitrogen-fixing plants could benefit growth by nitrogen cycling among the host legume plant, the soil, and associated plants growing on a particular site. Annual nitrogen-fixation rates of up to 200 kg/ha were reported from subterranean clover (*Trifolium subterraneum* L.) production under longleaf pine (*Pinus palustris* Mill.) stands in southern Mississippi (Hagedorn and others 1980). Supposedly, about 80 percent of the fixed nitrogen in legume pastures is transferred to the soil as plant and animal residues (Date 1970). Southern pines regenerated in subterranean clover pastures are potential beneficiaries of nitrogen-fixation by the clover. Nitrogen can be removed from the site through timber harvesting, forage grazing, and soil leaching (Haines and DeBell 1979).

In the sandhills of South Carolina near the Savannah River Project, common sericea lespedeza (*Lespedeza cuneata* [Dum.-Cours.] G. Don) apparently fixed nitrogen in soils to the point of increasing foliar nitrogen contents of sand pine (*P. clausa* [Chapm. ex Engelm.] Vasey ex Sarg.) by the third growing season (Jorgensen 1985). Similar results by the fourth growing season were obtained in loblolly pine (*P. taeda* L.) using common sericea lespedeza, "Caricea" lespedeza (an improved variety of common sericea), and Thunberg lespedeza (*L. thunbergii* [DC.] Nakai) in North Carolina where stemwood volumes were greater on the lespedeza treatments (Schoeneberger and Jorgensen 1989).

The purpose of this study was to determine whether subterranean clover, a cool season legume, fixes nitrogen in tame pastures sufficiently for it to be incorporated into the soil and subsequently translocated into associated loblolly and slash (*P. elliottii* Engelm.) pines. Specifically, this agroforestry study focused on comparing nitrogen content of the pine foliage from trees growing in tame pastures with and without subterranean clover. These preliminary results may reflect the value of subterranean clover in nutrient cycling and subsequent pine growth when used in agroforestry.

STUDY METHODS

The study area was located in a 4-ha tame pasture within the southeast range unit, Palustris Experimental Forest, McNary, Louisiana. The Unit was cleared in 1977; in the fall of 1980, the pasture was burned, disked, fertilized, and planted to subterranean clover (Pearson and others 1990). Clover seed were inoculated with *Rhizobium trifolii* at the time of planting (Hagedorn and others 1983). In the late summer of 1983 and subsequent years, randomly selected 0.4 ha paddocks were disked and treated with herbicide (ROUNDUP^R: isopropylamine salt of N-(phosphonomethyl gylcine)) to remove the summer, warm season growing grasses and to encourage fall and winter production of subterranean clover. During the ensuing winter, a third treatment paddock (the control) was established (subterranean clover removed from the paddock by applying herbicide during early March). Each year during late August or early September, 53 kg/ha each of P_2O_5 [23 kg/ha elemental phosphorus (P)] and K_2O [44 kg/ha elemental potassium (K)] were top dressed over all paddocks.

At the time of control treatment, loblolly and slash pine seedlings (0-1) were planted in all three treatment paddocks. Three rows of seedlings of each pine species were planted within each 0.4-ha paddock; each row served as replication for the chemical analyses. Pines were planted 1.3 m within rows, which were 6.7 m apart; pine density was about 1,165 seedlings/ha. Each replication contained 45 seedlings for each pine species.

Pine seedling heights were measured after the second and third years, and both heights and diameters were measured at tree age 5. However, the fifth-year tree measurements are not reported in this paper, since analyses are not complete. By the end of the third growing season, no differences in tree heights were noted (unpublished data).

Following the 1987 (samples collected May 1988) and 1988 (samples collected January 1989) growing seasons, composite pine foliar samples from 10 trees within each 45-tree replication row were collected. Foliage was analyzed for tree age (4- and 5-year-old trees), foliar location (upper and lower one-third of the tree crown vertical height), stage of growth (mature vs. immature needles), cultural treatments (none, disking, and chemical application), and species (loblolly and slash pine). Nutrient analyses, including nitrogen, phosphorus, and potassium, were conducted at the Alexandria Forestry Center, Pineville, Louisiana. The pine foliar samples were digested in sulfuric acid with copper sulfate added as a catalyst. Nitrogen was determined using an ammonia-specific electrode (Powers and others 1981), phosphorus was determined colorimetrically (John 1970), and potassium was determined by atomic absorption spectrophotometry. Soils on the paddocks were analyzed during August 1985, by the Feed and Fertilizer Laboratory, Louisiana State University, Baton Rouge. Although not used in these evaluations, averages for the soil analyses on the different cultural treatments are provided for background information (table 1).

Percentage of ground cover of subterranean clover was estimated during the spring each year from 20 plots (0.22 m^2) systematically located every 5 m apart on 2 diagonal

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Table 1.--*August 1985 soil analyses on different cultural treatments*

Attribute	Cultural Treatments		
	Control	Disk	Chemical
Organic matter (percent)	2.2	2.7	2.0
P (mg/kg)	*	*	5
Na (mg/kg)	11	13	10
K (mg/kg)	47	30	22
Ca (mg/kg)	1323	974	1216
Mg (mg/kg)	46	60	43
pH	6.8	6.0	6.7

*Missing data

transects across each quarter of a 0.4-ha paddock for a total of 80 estimates/paddock. A yield-ground cover equation, developed by double-sampling (Pearson and others 1990), was used to estimate subterranean clover yields:

$$Y = 49.1\ C$$

where Y is kg/ha ovendry yield and C is percentage of ground cover; the standard error of estimate was 1,505 kg/ha and the coefficient of determination (r^2) was 0.91.

RESULTS AND DISCUSSION

Subterranean clover yields during spring averaged 4,040 and 3,457 kg/ha on the disk and chemical cultural treatments, respectively (table 2). The control paddock had an average residual clover yield of 380 kg/ha; however, a high proportion of this yield during the latter years was volunteer white clover (*T. repens* L.), possibly transported by cattle grazing other areas and the study paddocks. Crude protein content of the subterranean clover during winter and spring varied from 18 to 23 percent (Pearson 1983); phosphorus content varied from 0.20 to 0.27 percent during the same period (Pearson and Barnett 1984).

Immature growing pine needles collected during spring had higher nitrogen content than mature needles on 4-year old pines regardless of species (table 3). Loblolly pine needles had higher nitrogen content than slash pine needles for both immature and mature stages of growth. Apparently, loblolly pines utilize nitrogen from the soil more efficiently than slash pines or at least translocated more nitrogen in the plant to the needles. Phosphorus and potassium content of the needles responded similarly to maturity and species--immature needles had higher analyses than mature needles, and loblolly pine needle analyses were higher than slash pine.

Needles in the upper one-third of the crown had more nitrogen than needles in the lower third for the 1987 (fourth) growing season but were not different for the 1988 (fifth)

Table 2.--*Subterranean clover yields (kg/ha) in the spring on different cultural treatments*

Treatment	Year						
	1984	1985	1986	1987	1988	1989	Average
Control	252	*	*	*	437	452	380
Disk	4188	4419	4910	4051	3457	3216	4040
Chemical	3953	4483	4910	3314	1841	2239	3457

*No measurement made.

Table 3.--*Chemical content (mg/g) of immature and mature pine needles (1987)*

Growth Stage	Nitrogen		Phosphorus		Potassium	
	Loblolly	Slash	Loblolly	Slash	Loblolly	Slash
Immature	16.6 a*	12.1 a	3.0 a	2.4 a	10.6 a	8.7 a
Mature	13.2 b	9.7 b	1.7 b	1.5 b	4.6 b	4.0 b

*Different letters in same column denote significance at the 0.05 level.

growing season (table 4). Nitrogen translocation from the lower needles possibly occurred prior to the May sampling but not by the January sampling. Nitrogen content of loblolly pine needles was higher during both sample years when compared to slash pine needles. Phosphorus and potassium contents of the needles were higher in the upper third compared to the lower third of the crown for both species in both years.

Although high yields of subterranean clover occurred on the tame pasture paddocks with the disk and chemical cultural treatments, nitrogen contents of pine foliage were apparently not affected prior to the 1988 growing season (table 5). However, nitrogen contents of needles were higher in both the disk and chemical treatments compared to the control treatment after the 1988 growing season (5-year-old trees). Loblolly pines in subterranean clover pasture had the highest nitrogen content in the needles with the chemical cultural treatment that exceeded amounts measured in either the disk or control treatments. Both disk and chemical treatments were similarly higher in nitrogen than the control for slash pine. Apparently, nitrogen fixation by the subterranean clover was sufficient to be incorporated into the soil and translocated to the pine needles during the fifth growing season. This higher nitrogen uptake by pines should eventually increase tree growth similar to results reported in North Carolina using lespedeza (Schoeneberger and Jorgensen 1989).

Phosphorus contents of the needles were not different among treatments in the 4-year-old loblolly pine or the 5-year-old slash pine (table 5). On the other hand, both the 1988 loblolly and 1987 slash pine needles were different among cultural treatments with the control higher in slash pine and the chemical treatment higher in loblolly pine. Except for the 1988 loblolly pine foliar analyses, potassium content of needles was higher on the control treatment than on either the disk or chemical cultural treatments. Possibly potassium in subterranean clover was not being recycled sufficiently to be translocated to the pines. Apparently, nitrogen and phosphorus were sufficiently cycled on the cultural treatments for absorption and translocation by the pines; hence, higher foliar nutrient contents on the disk and chemical treatments compared to the control treatment.

CONCLUSIONS

High yields of subterranean clover for forage are possible on tame pastures in the South where cultural treatments control the warm season grasses. Also, subterranean clover fixes nitrogen sufficiently to be absorbed and used by southern pines. The soil nitrogen apparently increases through nitrogen fixation by the cool season legume subterranean clover, but the nitrogen is absorbed and translocated by the pines only after several years of nitrogen fixation and accumulation in the soil. Immature, actively growing pine needles during spring have higher nitrogen,

Table 4.--*Chemical content (mg/g) of pine foliage from different location*

Crown Location	Nitrogen				Phosphorus				Potassium			
	Loblolly		Slash		Loblolly		Slash		Loblolly		Slash	
	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988
Upper third	16.7a*	16.1a	11.3a	11.2a	2.8a	1.4a	2.1a	1.1a	9.3a	5.9a	7.9a	5.2a
Lower third	13.1b	15.9a	10.5b	12.2a	1.9b	1.3b	1.7b	1.0b	5.9b	4.8b	4.8b	4.0b

*Different letters in same column denote significance at the 0.05 level.

Table 5.--*Chemical content (mg/g) of pine foliage from different cultural treatments for subterranean clover pasture*

Cultural treatment	Nitrogen				Phosphorus				Potassium			
	Loblolly		Slash		Loblolly		Slash		Loblolly		Slash	
	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988	1987	1988
Control	14.2a*	13.4c	11.3a	9.8b	2.4a	1.3b	2.0a	1.1a	8.3a	5.6a	7.5a	5.3a
Disk	14.8a	16.3b	11.0ab	12.2a	2.3a	1.4b	1.9ab	1.0a	7.2b	5.2a	5.8b	4.2b
Chemical	15.6a	18.4a	10.3b	13.1a	2.3a	1.4a	1.9b	1.1a	7.3b	5.2a	5.7b	4.4b

*Different letters in same column denote significance at the 0.05 level.

phosphorus, and potassium contents than mature needles. Needles from the upper crown generally have higher nutrient contents than lower crown needles. Loblolly pines apparently assimilate nitrogen more efficiently than slash pines.

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A Method for the Detection of Trace Quantities of Ethylene in the Atmosphere

Lewis R. Brown, Gerard S. Pabst, Jr.,
James L. Spencer, Jr., and Ted H. Filer, Jr.

INTRODUCTION

Ethylene is a natural product of metabolism in plants and is considered a plant hormone. When a plant is stressed, however, ethylene production increases significantly. Since stress ethylene is produced prior to the visual manifestations of stress, its early detection could make it possible to eliminate the cause of the stress, thereby reducing damage to the plant.

The most common method of detecting ethylene in the atmosphere is by gas chromatography (GC), and this method has been employed in our laboratory for several studies involving the microbial production of ethylene. However, all these studies were conducted using closed vessels and, as a consequence, the ethylene concentration in the atmospheres reached levels easily detected by GC. The sample size employed for the GC analyses was 100 μL, and with this sample size, a concentration between 0.13 and 1 μl was detectable using a thermal conductivity detector. Concentrations of ethylene of the above magnitude would not be expected in a field situation nor would adapting a gas chromatograph for field use seem practical.

This paper describes the evaluation of two methods amenable to field use to detect low levels of ethylene in the atmosphere.

METHOD ONE

An attempt was made to develop an ethylene detection system based on the reaction between ethylene and bromine water. This reaction decolorizes the bromine water and can be conveniently monitored by spectroscopic methods. Saturated bromine water, containing 3.35g bromine per 100g water at 25 °C, was obtained from American Scientific Products, Inc. A Bausch and Lomb Spectronic 20 Spectrophotometer was employed for measuring light transmittance.

Saturated bromine water was diluted 1:33 with distilled water, and samples were analyzed for percentage of light transmission at various wavelengths. Representative data are given in figure 1. This experiment was repeated using a 1:100 dilution of saturated bromine water in distilled water, and representative data are given in fig. 2.

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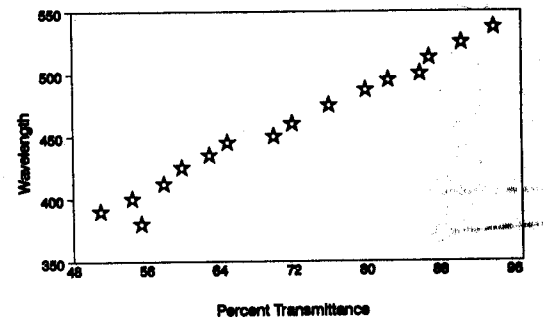

Figure 1.--*Spectrophotometric observations of 1/33 dilutions of bromine water using a wide range phototube and filter.*

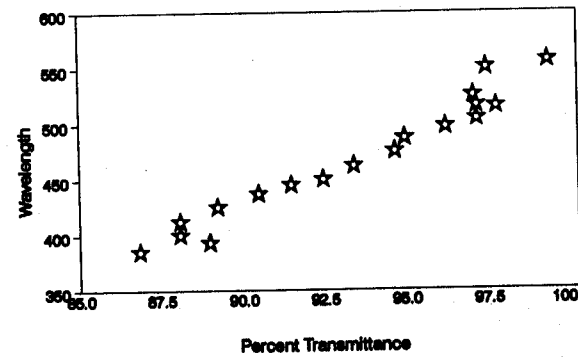

Figure 2.--*Spectrophotometric observations of 1/100 dilutions of bromine water using a wide range phototube and filter.*

Six ml of a 1:33 dilution of saturated bromine water in distilled water was pipetted into each of a number of spectrophotometer cuvettes, the light transmission measured (450 nm), and the contents transferred to a serum-stoppered, 16 x 100 ml glass test tube. A known amount of ethylene was injected into each tube, the contents of the tube mixed on a vortex mixer for 30 seconds, and the light transmission of the bromine water again measured. Representative results, as shown in fig. 3, indicate that the intensity of the color of the bromine water is reduced in proportion to the amount of ethylene injected into the tube.

Other tests were conducted by bubbling different amounts of air that varied in ethylene concentration through different concentrations of bromine water. The major problem encountered was that air without ethylene decolorized the bromine water, albeit at a rate considerably slower than that caused by ethylene. This problem was overcome by accounting for the decolorization caused by a given volume of air. It was found also that light accelerated decolorization slightly. Shielding the bromine water from light easily overcame this problem.

While the system did work, it was found that at very low levels of ethylene, where large volumes of air must be passed through the bromine water, the problem of decolorization by the air alone caused major problems. The controls necessary

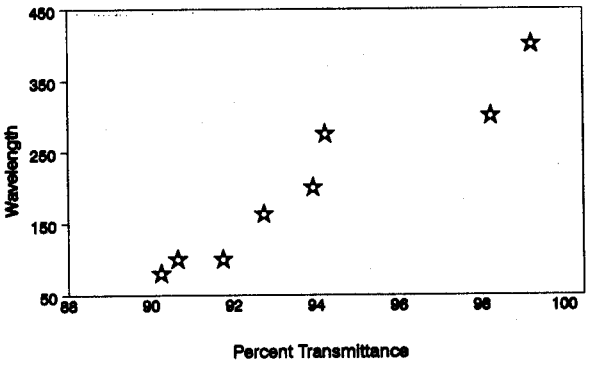

Figure 3.--*Spectrophotometric observations of 1/33 dilutions of bromine water at 450 nm after injection of ethylene.*

to obtain accurate results seemed to make the system impractical for routine use.

Obviously, the degree of decolorization is dependent upon (1) the concentration of the bromine water, (2) the volume of bromine water employed, and (3) the amount of ethylene added. It is realized, of course, that materials other than ethylene will decolorize bromine; in fact, any gaseous olefin will do this. The probability of the presence of other interfering gaseous compounds in the atmosphere being monitored is sufficiently remote and should not be a major problem. Even if such compounds did get into the atmosphere, they would cause a "false positive" reading, thereby indicating "stress" in the plants where, in fact, this might not be the case. The above situation is preferred to one where a false negative would fail to indicate stress even though it were present.

METHOD TWO

As stated earlier, gas chromatography is an accurate and reliable method of determining ethylene in air, but unfortunately, the concentration of ethylene expected is below the limits of detection of the gas chromatograph. Therefore, it was necessary to concentrate the ethylene in a sample in order to detect it by GC. Recently, Supelco, Inc., began marketing a thermal desorption unit (TDU) that makes collection of trace contaminants in air practicable. In the present case, the ethylene is adsorbed onto Carbotrap/Carbosieve contained in a small glass column, with the subsequent elution (thermally) of the gas from the column in the TDU.

This instrument thermally desorbs the gas and delivers it directly into the gas chromatograph for conventional detection.

Collection of the Sample. Samples were collected using an E. I. DuPont De Nemours and Co., Inc., Permissible Air Sampling Pump, Model P-4000. The pump is set for the desired airflow, the 4 mm I.D. Carbotrap/Carbosieve 200 desorption tube (Supelco, Inc., 2-2042) is inserted into a rubber tube connected to the pump, and the pump turned on. Once the sample has been collected, the desorption tube is removed from the rubber tubing and placed in a carrying vial (Supelco, Inc., 2-0375).

Thermal Desorption Unit and Gas Chromatograph. The Thermal Desorption Unit is illustrated in figure 4 and is connected to a Fisher Model 1200 Gas Partitioner.

Settings on the TDU. The TDU set for use with the GC should have a helium gas flow of 60 psi with the tube heat selector switch (fig. 4) set on automatic, the tube heat timer set for 10 minutes, and the tube heat set at 330 °C.

Settings on the Gas Partitioner. The GC should have the following settings and conditions. The helium gas flow should be maintained above 15 psi to maintain the cutoff relay (this is necessary because the column is connected directly to the TDU transfer line). The column is a 10-foot, 1/8-inch stainless steel column packed with 100/120 mesh Carbosieve S-II. The column temperature is 150 °C. The Bridge current is 175 mA with an attenuation of 1x. The Fisher Recordall, Model 5000 is connected to the GC and is set to record at 0.25 cm/min at 0.001 volts full scale.

Desorption of Ethylene Trapped on Desorption Tubes. The desorption tube is placed in the tube desorption chamber, the valve arming button is pressed, and the valve handle moved to the heat position.

Determination of Ethylene. To determine the volume of ethylene injected directly into the TDU, an empty desorption tube was placed in the tube desorption chamber of the TDU, the valve arming button was depressed and the sample injected through the tube desorption chamber injection port. Immediately (within 10 seconds) the valve handle was turned to heat.

To develop a curve for ethylene, 5, 10, 25, 50, and 100 μl samples of ethylene gas mixture were made up to a total volume of 100 μl with air and injected into the TDU as described above. Ethylene was detected by comparison of retention time of the unknown with the retention time of a known sample of ethylene. Quantification was accomplished by comparison of the area under the peak with the area under the peak of a known amount of ethylene. The results are shown in fig. 5 by the solid dots.

Detection of Ethylene Trapped on Desorption Tubes. With desorption tubes attached to the DuPont air pump, various amounts of an air mixture containing ethylene were passed through the desorption tube. The tubes were then desorbed by placing a tube into the desorption chamber of the TDU, depressing the valve arming button, and immediately

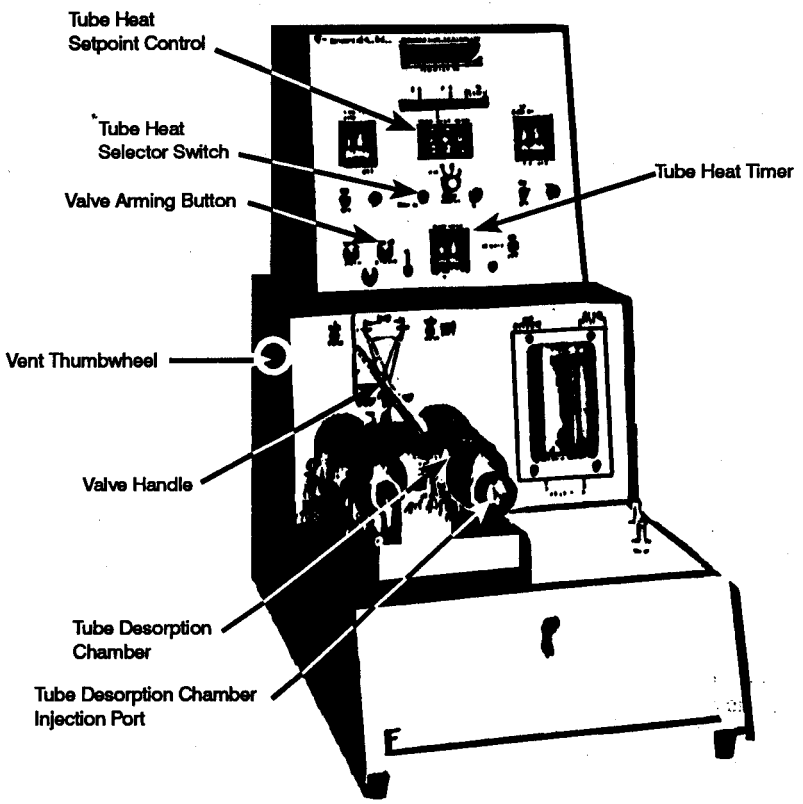

Figure 4.--*Diagram of the thermal desorption unit.*

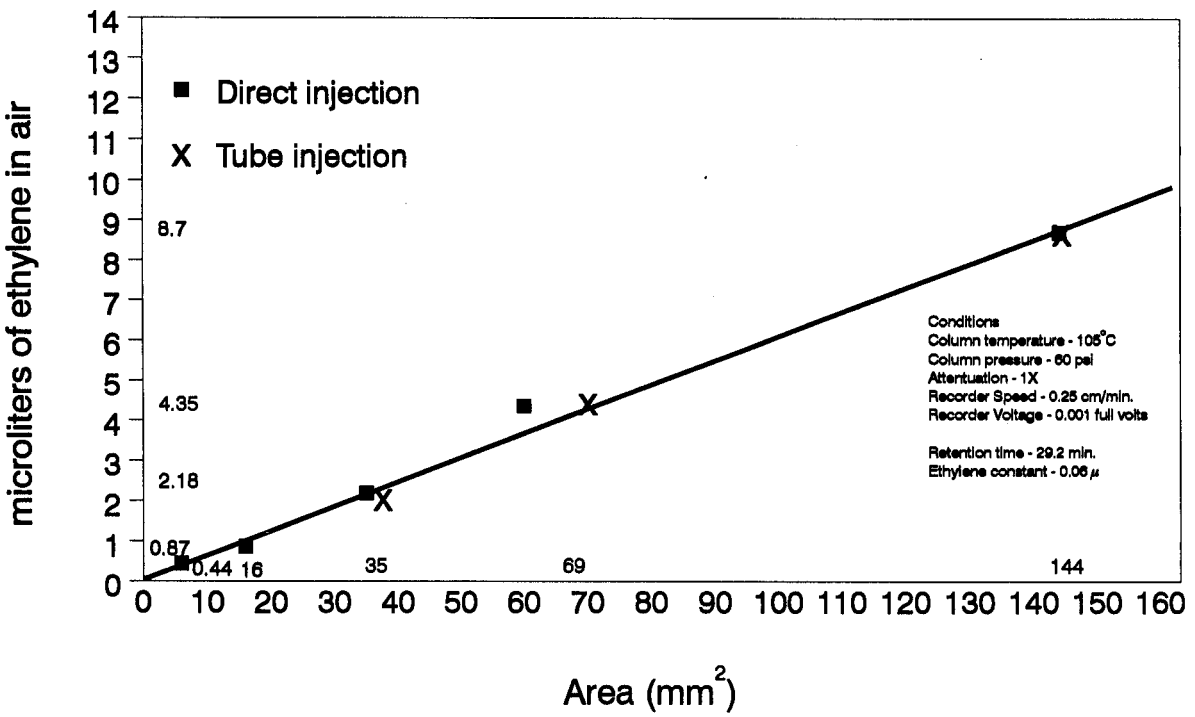

Figure 5.--*Comparison of directly injected ethylene with thermally desorbed ethylene.*

moving the valve handle to the heat position. After desorption is indicated on the GC recorder, the valve handle was moved to the load position for subsequent tubes to be desorbed.

The results of desorption of the ethylene from the tubes and the resultant detection of the ethylene are shown by x's fig. 5. As may be observed, the results of this experiment e essentially identical to those achieved by injecting ylene directly into the TDU, thus indicating that, within he limits of detection, all of the ethylene trapped on the lesorption tube was recovered.

While the system worked well, very low concentrations of ethylene in air could not be detected due to (1) the lack of sensitivity of the thermal conductivity detector and (2) the ow breakthrough volume for the desorption tubes. The smallest amount of ethylene detectable using the TDU was 1 µl, and the breakthrough volume for the desorption tubes was 100 ml/min for 8 minutes or 10 ml/min for 2 hours. Under these conditions, the ethylene had to be in a concentration of at least 0.8 ppm or 800 ppb. The sensitivity can be increased by at least a hundredfold through the use of a flame ionization detector, making it possible to detect 8 ppb.

The thermal desorption unit is designed in such a way as to make it possible to focus the ethylene from the 4 mm (I.D.) collection tube onto a smaller (2 mm I.D.) desorption tube. It was found that the ethylene from three 4-mm collection tubes could be focused onto a single 2-mm tube, thereby making it possible to detect 0.26 ppm with the thermal conductivity detector. The flame ionization detector, therefore, should be able to detect 2.6 ppb.

ACKNOWLEDGMENTS

This project was supported in part by U.S. Department of Agriculture, Forest Service.

Optimization of Graphite Furnace Atomic Absorption Technique for Determination of Rubidium in Southern Pine Beetle, *Dendroctonus frontalis*, for Mark-Recapture Studies

W.T. Thoeny, A.E. Tiarks, and M.L. Elliott-Smith

Little is known about the dispersal of the southern pine beetle, *Dendroctonus frontalis* Zimm. (SPB) and the role it plays in the population dynamics of this important pest of southern pines. Despite the importance of scolytid bark beetles, few mark-recapture studies have been conducted with any species. This lack of information is partly due to the lack of a suitable marking technique for these small insects. Moore and Taylor (1976) demonstrated a 32p marking technique for the SPB, but this method has been abandoned due to safety and environmental concerns. The use of trace elements, particularly rubidium (Rb), as internal markers for insects has been widely adopted (Bridges and others 1989 and references therein). Labelling of phytophagous insects has commonly been accomplished by incorporating trace elements into artificial diets (Stimmann and others 1973) or by application and translocation through host plants (Van Steenwyk and others 1978, Bridges and others 1989). Analyses by atomic absorption spectroscopy have demonstrated that addition of low levels of Rb to larval diet of several insect species does not affect longevity, fecundity, or behavior.

Rubidium occurs naturally at low levels in soils, and natural background levels are normally low in native flora and fauna. Previous rubidium-marking experiments have established a criterion for the level of Rb necessary for the insect to be considered a marked individual (Stimmann 1974). This standard based on the mean background Rb level plus 3 SD results in a 99-percent confidence limit that a marked insect did not belong to the natural population (Bain and Engelhardt 1987).

The inherently low background Rb levels and small size of the SPB necessitate the use of graphite furnace technology. With the advent of the platform furnace technique and control of experimental conditions, the graphite furnace has become a convenient analytical tool, suitable for routine analysis of inorganics, although little has been published on the determination of Rb (Slavin and others 1983). During graphite furnace analysis, desolvation of the sample, dissociation from the matrix, and generation of analyte ground state atoms occur sequentially during predetermined drying, pretreatment, and atomization steps. Before analyzing samples containing complex unknown compositions, it is essential to determine whether the recommended standard conditions for the element in question are appropriate, or whether alterations are necessary for optimum performance (Perkin-Elmer 1985). Grobenski and others (1983) reported on optimum conditions for Rb analysis and determined a detection limit of 5 pg, with no ionization effect. They recommended a thermal pretreatment temperature of 800 °C and a fast heating atomization step to 2,000 °C for routine analysis with the L'vov platform in pyrolytically coated graphite tubes. The purpose of this study was to establish optimum operating conditions for Rb determination of unmarked SPB by atomic absorption spectroscopy (AAS) using a graphite furnace.

MATERIALS AND METHODS

Adult beetles were obtained from naturally infested loblolly pines, *Pinus taeda* L., within the Kisatchie National Forest in central Louisiana. Infested bark samples were placed into rearing containers, and emerging beetles were collected for analysis. Beetles were killed by freezing and dried for 24 hours at 70 °C before determining dry weights. Fifty beetles were placed in 8-ml borosilicate glass vials along with 1.0 ml reagent nitric acid (HNO_3) and heated at 100 °C for 4 hours until the samples cleared and diluted to 25 ml with deionized water.

NBS pine needle standards (ref. mat. 1575) were prepared by ashing 0.5 g of needle sample in a muffle furnace at 500 °C and placing 0.02 g of ash into 100-ml volumetric flasks. Preliminary results indicated that perchloric acid was interfering with Rb analysis, so ash was diluted to 100 ml with 0.2 percent HCl in deionized water and 0.2 ml HNO_3 or with both 0.2 ml HNO_3 and 2 ml $HClO_4$.

Samples were analyzed by atomic absorption spectroscopy using a Perkin-Elmer 2100 with an HGA 700 graphite furnace and AS-70 autosampler (Perkin-Elmer Manufacturers, Norwalk, Connecticut). An electrodeless discharge lamp (wavelength 780 nm) was used to detect rubidium. Samples were atomized on L'vov platforms within pyrolitically coated graphite tubes. Rubidium concentration was determined by the area under the peak of the AAS signal.

Optimum pretreatment temperature was determined by setting the dry and atomization parameters to the optimum values taken from the recommended conditions (Perkin-Elmer 1985). Sample aliquots (20 μL) of RbCl sample solution (10 μg/L) and SPB-acid matrix solution were analyzed at thermal pretreatment temperatures of 200, 400, 600, 800 (recommended condition), 900, 1,000, 1,200, and 1,400 °C.

Optimum atomization temperature was determined by setting the dry and pretreatment parameters to the optimum recommended conditions and analyzing aliquots of RbCl sample solution atomized at 1,000, 1,200, 1,400, 1,600, 1,700, 1,800, 1,900 (recommended condition), 2,000, 2,100, and 2,200 °C. Based on these results, the SPB-acid matrix

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solution was analyzed at atomization temperatures from 1,600 - 2,200 °C at 100° increments.

In some instances, standards that are sufficiently similar to the sample solution are difficult to prepare because of the presence of matrix materials or solids (Perkin-Elmer 1985). The standard additions technique can often be used to correct for these nonspectral interferences by determining the concentration of the analyte. Standard additions were conducted with the SPB-acid matrix solution and NBS pine needle samples in HNO_3 or in HNO_3 plus $HClO_4$.

The standard additions technique was used to check for nonspectral interferences by adding increasing volumes of a standard to a constant volume of unknown. Standard additions were conducted using SPB-acid matrix solution, ashed pine needle samples in HNO_3, or ashed pine needle samples in HNO_3 plus $HClO_4$ as the unknown. A five-μL aliquot of the unknown was added to the furnace followed by a 15-μL aliquot of blank plus standard. The amount of standard was increased from 0 to 10 μL in 1-μL increments. Linear regression analysis was used to find the relationship between Rb added as RbCl and absorbance.

It is often necessary to add a chemical matrix modifier that allows higher pretreatment temperatures in order to remove matrix compounds that interfere with the analyte signal. Little has been published on the determination of Rb with the graphite furnace, and Slavin (1984) does not include established matrix modifier recommendations for Rb. However, in order to determine if matrix interferences existed, two commonly used matrix modifier compounds were evaluated. A 4- percent solution of ammonium phosphate ($NH_4H_2PO_4$) and a l-percent solution of magnesium nitrate ($Mg(NO_3)_2$) were evaluated for matrix modifier benefit with NBS pine needle standard samples in HNO_3 and HNO_3 plus $HClO_4$.

RESULTS AND DISCUSSION

Optimum thermal pretreatment temperatures for Rb in both the RbCl sample and the SPB-acid matrix sample were determined to be the recommended standard condition of 800 °C (fig. 1). Although the Rb signal did not decline significantly at 900° or 1,000°, Grobenski and others (1983) reported minor losses at 900°. Because of this, a pretreatment temperature of 800 °C will be used for analyzing Rb in SPB during our research. An atomization temperature of 2,000° was determined to be the optimum condition for Rb analysis in both the RbCl sample and the SPB-acid matrix sample (fig. 2). This 100° increase in temperature resulted in a significant increase in Rb signal from the recommended condition of 1,900° and was also recommended by Grobenski and others (1983).

When possible, standards should be made up in a matrix similar to the samples to minimize the possibilities of nonspectral interferences. In some instances, standards that are sufficiently similar to the sample solution are difficult to prepare because of the presence of matrix materials or solids

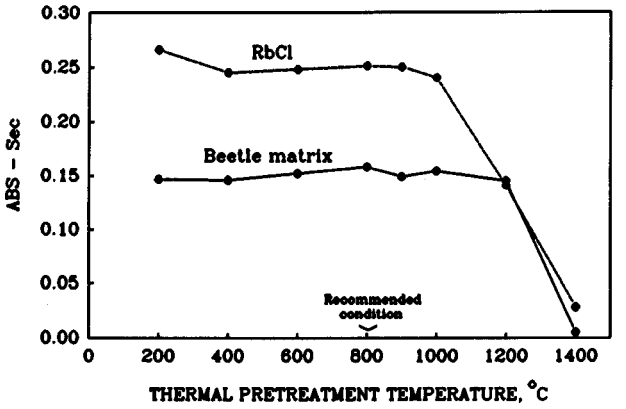

Figure 1.--*Optimization of pretreatment temperature for RbCl sample and digested southern-pine-beetle-acid matrix sample by atomic absorption spectroscopy with a graphite furnace.*

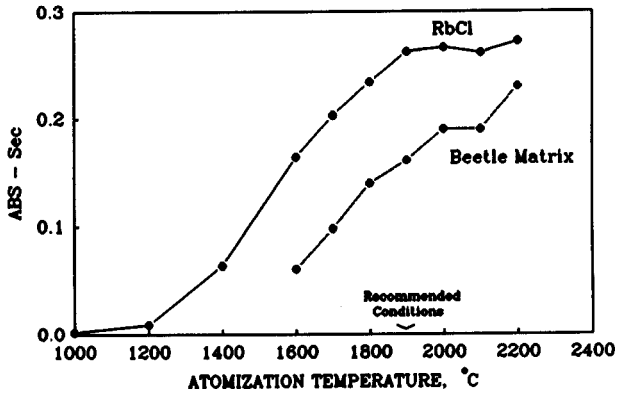

Figure 2.--*Optimization of atomization temperature for RbCl sample and digested southern-pine-beetle-acid matrix sample by atomic absorption spectroscopy with a graphite surface.*

(Perkin-Elmer 1985). In our case, insufficient knowledge about the constituents of the beetle matrix and the materials that may cause interferences in measuring Rb prevented us from duplicating the sample matrix. The possibilities of nonspectral interferences were examined by checking the recovery of added standard to a constant volume of unknown. We found a linear relationship between the combined absorbance of the sample and the increasing volume of standard solution indicating interferences are not a problem (fig. 3). The intercepts of the lines are close to the concentration of the sample when no Rb was added, showing that any interferences are consistent across the Rb concentrations.

However, the solution used to dissolve the needle ash had a major influence on the recovery of added Rb. When the ash was in 0.2 percent HNO_3, the recovery of added Rb was 89 percent. When the solution was 0.2 percent HNO_3 plus

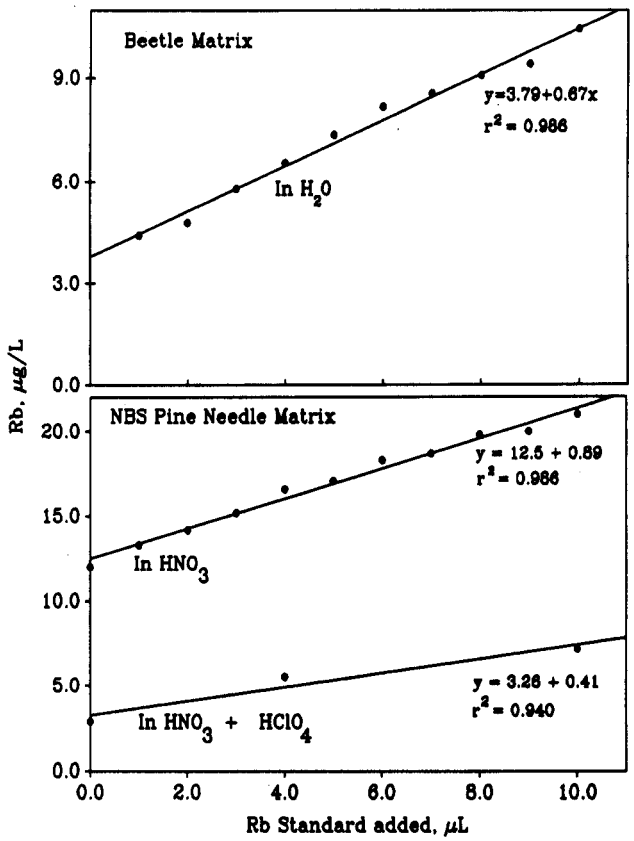

Figure 3.--*Standard additions technique with RbCl standard solution (20µg/L) to southern-pine-beetle-nitric-acid matrix sample, NBS standard pine-needle-nitric-acid matrix sample, and NBS standard pine-needle-nitric-plus-perchloric-acid matrix sample.*

2 percent $HClO_4$, the recovery dropped to 41 percent. The presence of perchloric acid in the sample reduced recovery by half. Rubidium perchlorate ($RbClO_4$) decomposes when heated (Weast 1975), and some Rb is evidently lost in one of the earlier drying or pretreatment steps when $HClO_4$ is added.

The concentration of nitric acid also affected the recovery of Rb from the beetle matrix. Because the sample was brought to volume with water rather than nitric acid, the recovery of added Rb was only 67 percent. Thus, the kind and concentration of the acid used to dilute the samples are critical. The standards and samples should be made in the same solution, and any dilutions of the samples or standards need to be made in that solution and not water.

There was no improvement of the Rb signal with the addition of ammonium phosphate as a matrix modifier. Although there was a slight improvement with magnesium nitrate, contamination problems with this compound negated any advantage.

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Chemical Stimulation of Host Tree Antibiosis for Southern Pine Beetle, *Dendroctonus frontalis* Zimm., Suppression

M. C. Miller, T. G. Rials, and D. N. Kinn

INTRODUCTION

The southern pine beetle (SPB), Dendroctonus frontalis Zimmermann, is responsible for most insect-caused mortality of timber in the South. Periodic epidemics produce timber and pulpwood losses valued in the millions (Price and Doggett 1978). Current methods for slowing or stopping SPB spots--cut and leave, use of insecticides, pile and burn, or intensive salvage (Swain and Remion 1981)--are limited by cost of chemicals, labor and equipment, or environmental concerns about pesticides (Billings 1980). Due to the potential hazard to humans, livestock, beneficial insects, fish, and wildlife (Bennett and Ciesla 1971), there will likely be future restrictions on use of chemicals. Alternative methods for protecting high value trees from SPB in an economical and environmentally sound manner are needed.

Induced host plant resistance may have potential as an alternative method for SPB suppression. Although resistance to pests and pathogens has been induced by chemical treatment of some plants, a response against insects has not previously been reported in treated trees (Stipes 1988). A formulation of 32.7 percent sodium N-methyldithiocarbamate (SMDC) in 99.9 percent dimethylsulfoxide (DMSO) (4:1 v/v) applied by hack and squirt appears effective against SPB (Roton 1987). This formulation induces a hypersensitive response in which long, vertical, necrotic lesions are formed. The response may change monoterpene composition, does not kill trees when properly applied, and is apparently not harmful to natural enemies (Roton 1987). SMDC is a soil fumigant for control of fungi, bacteria, nematodes, weeds, and soil insects (Thomson 1986). DMSO is an agricultural solvent, penetrant, carrier, and antiviral agent (Smale 1969).

The method of introducing the chemical into the target tree may influence treatment efficacy and acceptance. Infusion is less contaminating than spraying, requires less labor and equipment, and relies instead on atmospheric pressure and the tree's uptake/translocation capability (Stipes 1988). The research described here shows the residual life of SMDC and tests the ability of SMDC+DMSO infusion to inhibit SPB brood production. Also reported here is a preliminary evaluation of SMDC+DMSO infusion on SPB ratio of increase (Thatcher and Pickard 1964), a measure of the success of SPB development in individual trees.

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MATERIALS, METHODS, AND RESULTS

Residue Tests. In the fall of 1986, active SPB spots in Stewart County, Georgia, were used to evaluate residual activity of the test compound[1]. Four replications, each of 32.7 percent SMDC in DMSO (4:1 v/v) in hacks, bark hacks only, and no treatments (control), were applied to loblolly pines (*Pinus taeda* L.). The chemical (5.0 - 10.0 ml) was applied to 2.5 cm long by 2.0 cm deep axe frills made around the circumference of the tree at 1.0 m above the ground and bridged by 2 to 4 cm of undamaged bark. Uninfested trees were sampled 1.0 m above the infusion point, at midbole, and 1.0 m below the live crown. The outer xylem, phloem, and bark were split off from small portions of the trunk at the designated height. The bark was peeled away, phloem separated from xylem and cut into small pieces. The upper 1.0 cm of xylem was turned into wood shavings. Samples were immediately processed, frozen, and stored in freezers.

Extracts were made from the samples for analysis by gas chromatography (GC) for methylisothiocyanate (MIT), the breakdown product of SMDC. A 5-10 g sample of xylem shavings or phloem bits sat overnight covered with water at room temperature in tared liquid scintillation vials. The aqueous portion was salted, 5.0 ml of ethyl acetate added, and then gently shaken for an hour. The organic layer containing MIT was drawn off and dried with sodium sulfate. GC analysis was performed on a capillary column at 180 °C with a nitrogen-phosphorous detector at 275 °C. Residual chemical was quantified by comparison of integrator counts of samples to counts from an analytical standard curve. Total nanograms of MIT was obtained by linear regression of emergence on dose per application point and expressed as ppm MIT, sample dry weight.

In spring 1987, eight pines were treated with chemical and sampled for residues as in 1986. Samples were taken 1, 4, 7, and 14 days posttreatment. The test was repeated in late summer, deleting sample day 4 and adding day 21.

In spring 1988, six trees were treated with chemical. One tree was intensively sampled at 3 heights (low, mid, top) as described. Three replicates at each height of phloem strips and drilled xylem shavings were removed and placed on dry ice. Day 1 samples were taken at 1, 5, 10, and 24 hours. Subsequent samples were taken on day 4 and weeks 1, 2, 4, 8, 10, 12, 14, and 20. In addition, infusion sites were sampled on weeks 2, 4, 8, 10, 12, and 14. All residues were determined as described above.

[1]Berisford, C. W. and M. J. Dalusky. 1989. Efficacy of systemic toxicants for southern pine beetle control. Final Report, U.S. Department of Agriculture, Forest Service, SFES Cooperative Agreement #19-87-097. 11 p., 2 tables, 9 figures. 1989.

Results. The 1988 SMDC+DMSO mobility study (fig. 1) confirmed results from 1986 and 1987. No residues were detected above the low height samples. Residual MIT, detected in the xylem at 1.0 m above the application point at 5 hours posttreatment, was undetectable by day 28. Ploem residues were detected at 1.0 m at 10 hours, peaked at 24 hours, then tailed off. No residues were detected from application sites after day 21 through day 140. The streaking effect on the xylem extended to midbole by day 14 and into the upper bole by week 4. The absence of residues after 21 days suggests that the infused formulation may metabolize within, or volatilize from, the tree.

Efficacy tests with hanging bolts, 1987. Loblolly pines at the head of active SPB infestations (spots) were treated in Camp Livingston, Catahoula Ranger District, Kisatchie National Forest, Louisiana. From 1984-1987, 5.0 ml of chemical were placed in horizontal, 1.0 - 5.0 mm deep X 75 mm long, axe frills, 1.0 m above the ground. Six unattacked trees were felled on the same day 1.3, 12, 18, 20, 27.5, and 28 months posttreatment and each cut into four 150 cm lengths. Four bolts, each from the 12-, 20-, and 27.5-month trees were moved to Dawson County, Georgia, and, with control bolts, were hung from trees at the front of an active SPB spot (Berisford and others 1980). Trees supporting bolts were baited with frontalure to ensure attack. Bolts from the remaining trees and an untreated control tree were installed at the advancing edge of a SPB spot in Rapides Parish, Louisiana.

When SPB became pupae or callow adults, 41.0 cm sections of these bolts were caged to collect emerging beetles. When emergence ceased, gallery lengths were measured and oviposition estimates (eggs/m^2) calculated (Foltz and others 1976).

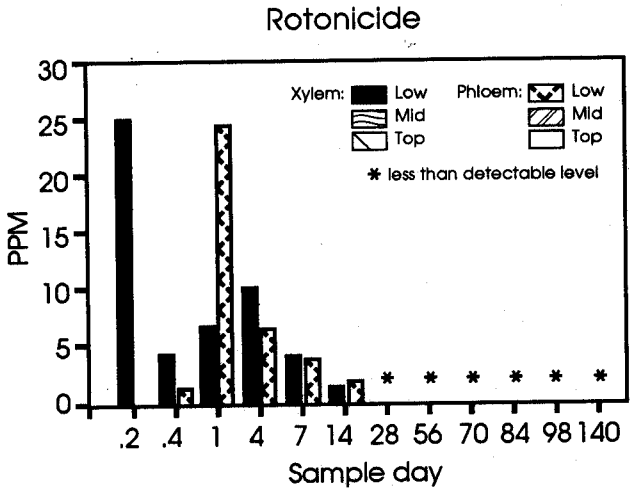

Figure 1.--*Residues of SMDC (MIT) detected in the xylem and phloem of treated pines at 1.0 m above the infusion point, spring - fall 1988.*

Results. In Louisiana, brood emergence from treated bolts was 58 percent less than controls. In addition, there was 54 percent less emergence from untreated bolts than support trees (fig. 2). There was only 3 percent variation in emergence from treated tree bolts. Estimated egg densities were similar in treated and check bolts, but fewer eggs were found in treated and check bolts than in support trees (fig. 2). There was less survival in treated bolts (fig. 3) and less survival with longer treatment-attack intervals. The Georgia replicates were misplaced, and it was not possible to analyze the data due to lack of experimental error. However, the emergence counts from each treatment were similar to those seen in the Louisiana test.

There was a consistent reduction in SPB emergence in Louisiana. Apparently SMDC+DMSO reduces brood production indirectly by eliciting a hypersensitive response. Fewer SPB emerged from trees treated from 1.3 to 28 months before felling. Therefore, the induced response can last more than 2 years. Lower survival in check bolts than support trees is probably due to desiccation of cut bolts.

Dose response/efficacy tests. Tests were conducted in active SPB spots consisting of 12 to 27 infested trees in the Bienville Ranger District, Bienville National Forest, Mississippi. From November 16-18, 1988, five unattacked trees at the head of each of six active SPB spots were randomly assigned to four treatments and a control. Chemical (3.0, 6.0, 9.0, or 12.0 ml) or water (12.0 ml) were

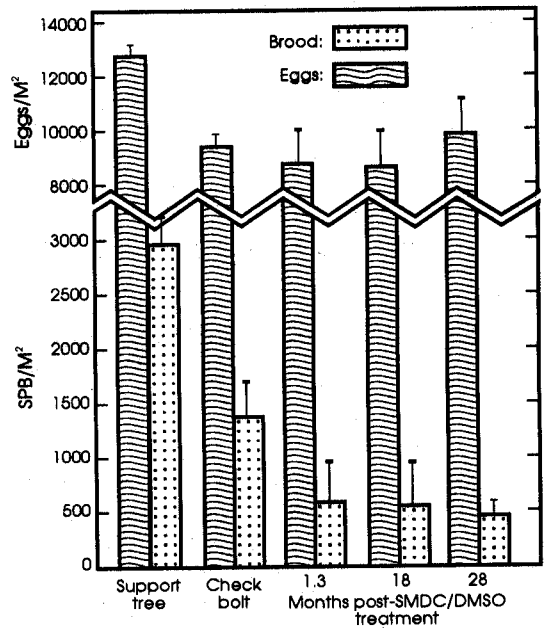

Figure 2.--*Southern pine beetle development; estimated oviposition and emergence counts from bolts from treated trees, untreated control bolts, and from bolts taken from support trees. Gates = + 2 SE.*

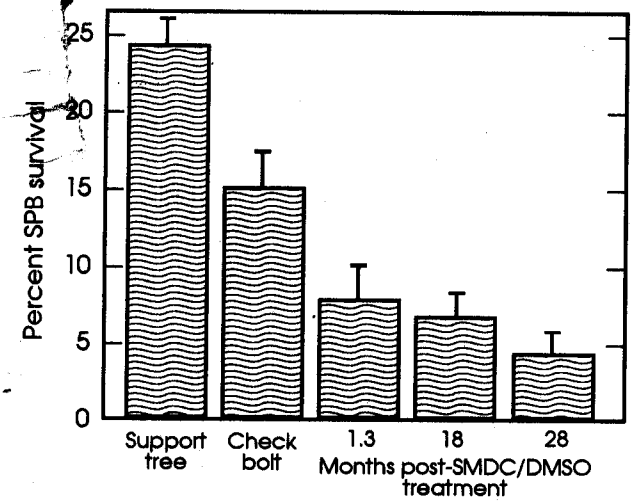

Figure 3.--*Percent survival of southern pine beetle in bolts taken from support trees, untreated check trees, and SMDC + DMSO-treated. Gates = + 2 SE.*

pipetted into horizontal axe frills, as described above. On February 21, 1989, frontalure was placed on trees to ensure SPB attack.

Bark samples (ca. 30 cm long) were removed from the circumference of the bole at 3.7 and 6.1 m when SPB reached the pupa/callow adult stage. Eight disks (94 cm^2) were removed from each 30-cm long sample for radiography, each placed in a rearing cup, and emerging SPB were counted. Data were analyzed for each sample height with polynomial regressions of emergence count means and weighted with the reciprocal of the variance. Significant regression of emergence on dose per application point was plotted to produce a dose-response line with 95 percent C.I.

Five loblolly pines (*Pinus taeda* L.) in Camp Livingston, Catahoula Ranger District, Kisatchie National Forest, Louisiana, were treated on May 23, 1989. The bark was shaved smooth, and 2.2-cm holes were drilled on 15.24-cm centers around the circumference of each tree. Coiled Tampax^R tampons were pushed into the holes and tamped even with the bark surface. Each tree received either 2.5, 5.0, or 10.0 ml SMDC+DMSO/hole injected through the center of the coiled plugs. There were two 10.0 ml/hole treatment trees in each block, control trees received 10 ml of water/hole, and infusion sites were wrapped with two layers of duct tape. The same method was used to infuse trees at the head of active southern pine beetle infestations of 40 or more trees on the Yellowpine Ranger District, Sabine National Forest, Hemphill, Texas, on May 30, and on the Neches Ranger District, Davy Crockett National Forest, Ratcliff, Texas, from June 5 - September 30, for a total of nine replicated trials. If SPB did not attack by posttreatment day 14, aggregation pheromone (frontalin: a-pinene 1:2) was applied to ensure attack. The data were processed as described above for the Mississippi studies.

Results. There were no significanct regression lines for emergence from Mississippi tests. While the mean response across doses and heights was fairly constant, the consistency of the response improved as dosage increased. The residuals of the observed and predicted values showed that the variance of the data decreased with increasing dose. Emergence counts were lower, and the range of the data was narrower from 6.0 and 12.0 ml/hack treatments compared with the check and other treatment trees.

In Louisiana and Texas, the regression lines for emergence from both heights were not significant when all plots were analyzed. However, when the data were tabulated, emergence was found to be high from all trees from the first three plots, in which beetle mass attack occurred 7 days posttreatment. In six later blocks, mass attack occurred between 14 and 21 days. In these blocks at the upper height, there was no significant dose-response regression. However, at the lower height, there was a significant decreasing linear response (Prob. $>F = 0.0001$, $r^2 = 0.84$) as dose increased. Confidence intervals (95 percent) showed significant reductions with increased dose. At the 10.0-ml infusion rate there was a 92-percent average reduction in SPB emergence (fig. 4).

Preliminary ratio of increase tests. Bolts (91.5 cm) were cut at 4.6 m from trees in two blocks installed on the Neches Ranger District, Davy Crockett National Forest, Ratcliffe, Texas, on June 6, 1989. Portions (41.0 cm) were excised from the larger bolts and placed in rearing cans.

Successful SPB attacks were identified and counted (Thatcher and Pickard 1964) on four bark disks (94 cm^2) cut from the lower portion of each bolt. The bark surface area of each bolt was measured, and all data were converted to counts per 0.1 m^2. The ratio of increase (RI) was calculated for each bolt using the total emergence from each bolt and the average number of successful SPB attacks from the four disk subsamples. The RI was derived from the formula (SPB

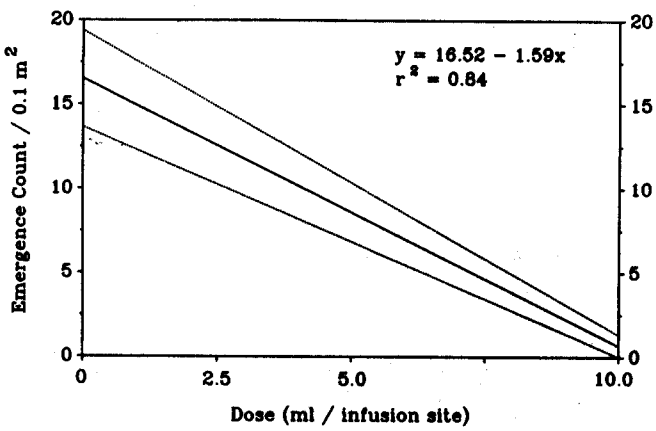

Figure 4.--*Linear regression of SPB emergence against SMDC + DMSO applied by the tampon in a hole method in six replicated in East Texas. The center line is the regression surrounded by 95-percent C.I.*

emergence) / (2 * number successful attacks) (Thatcher and Pickard 1964). Average SPB brood emergence and RI were tabulated.

Results. The ratio of increase for untreated east Texas SPB infested trees in July and August is 0.4:1 (table 1). The RI from treated trees is from 10.5- to 14-fold less than that of untreated infested trees, and corresponds with a 90 to 97 percent reduction in SPB emergence (table 1).

Infrared spectroscopy. The observed synergism of the SMDC+DMSO system prompted speculation concerning possible chemical reaction between the components. To resolve this question, the infrared spectum of an aged, SMDC+DMSO mixture was collected, and a direct subtraction of the DMSO spectrum performed. Infrared spectra of newly made SMDC and DMSO, of SMDC+DMSO, and of 3 month SMDC+DMSO mixtures, were collected with a Nicolet Fourier Transform Infrared Spectrophotometer (Model DXB-20). Samples were prepared as a smear on a KB^r pellet and data collected over 10 scans at a resolution of 4 cm^{-1}.

Results. When the resulting difference spectrum is compared with that of a fresh SMDC solution over the region 1,900 cm^{-1} to 900 cm^{-1} (fig. 5), some differences in peak intensities are readily apparent. However, the frequencies of the absorption bands are esentially identical. The slight shift to a higher frequency of the C=S peak (1,300 cm^{-1}) in the DMSO mixture may indicate some associative phenomenon between the components. There is no evidence to suggest formation of any new compound in the neat mixture.

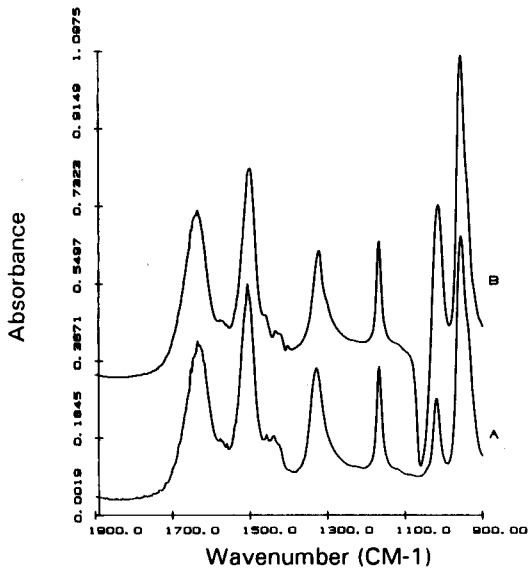

Figure 5.--*Comparison of the infrared spectrum over the region 1,900 cm^{-1} to 900 cm^{-1} of (A) a fresh SMDC solution, and (B) an aged, SMDC + DMSO mixture minus DMSO (difference spectrum).*

DISCUSSION AND CONCLUSIONS

In Georgia, mobility testing showed little or no movement of MIT above the lower bole, and then only at very low levels. Residues peaked within 3 days of application and were only present in the lower bole for 3 weeks. The residue studies do not support a SMDC+DMSO translocation hypothesis.

Table 1.--*Ratios of increase and emergence/0.1m^2 after treatment with various doses of SMDC+DMSO*

Dose	Ratio of Increase Mean	n	Emergence /0.1m^2 Mean	Percent Change
0	0.42:1	1	26.83	
2.5	0.04:1	2	1.08	-95.97
5.0	0.04:1	2	2.72	-89.86
10.0	0.03:1	4	0.77	-97.13

The induced response is at least partially responsible for inhibiting SPB brood development. Efficacy is a function of the application method and dose. In Mississippi, there was a nonsignificant reduction in emergence. In Texas, comparable doses applied with a tampon in a hole significantly reduced emergence. The Louisiana hanging bolt studies, showing reduced emergence, are supported by the Texas results. Treated trees can affect SPB emergence up to 28 months after infusion. Girdling of the tree by SPB during mass attack soon after treatment reduced efficacy.

SPB development is reduced by SMDC+DMSO infusion. The pitch-soaked lesion response is involved in inhibiting of SPB brood development. Direct toxicity of the mixture of active ingredients may not be the main source of mortality. The small amount of chemical infused into the host tree, the lack of mobility, and the short-term residues, suggest stimulation of defensive chemical production. The induced resinosis and streaking of the xylem appear to be moisture dependent. The tearing effect was present continuously under high moisture conditions in Louisiana and after heavy rainfall in Mississippi. Results are more effective as the period between SMDC+DMSO infusion and beetle attack lengthens.

Lack of available moisture for translocation of the chemical or early attack on the tree by SPB before development of pitch-soaked lesions may affect resinosis and length of lesions. Others have observed that residual MIT production from SMDC fumigation of transmission poles varies widely in different buffer solutions (Miller and Morrell 1989). Two of us (Miller and Kinn, unpublished) observed the absence of resinosis in trees treated under drought conditions and the start of resinosis 2 months later shortly after a period of heavy rainfall in the summer of 1988.

Residual MIT volatilizes at fungitoxic concentrations when wetted (Zahora and Morrell 1988). This suggests that in the living tree an active ingredient might remain immobile, or cells might not be activated, until sufficient moisture is available. The range of effects of residual MIT on living trees is not known.

Infusion of SMDC+DMSO may be an alternative to other suppression tactics. The hazard to the applicator and the user appears to be low. The LD_{50} of SMDC is 820 mg/kg, much less toxic than either Lindane, Chlorpyrifos, or Fenitrothion (Thomson 1986). DMSO assists in translocation of several insecticides, but did not markedly lower LD_{50} values of insecticides used against boll weevil (Moore and others 1970). Oligman (1965) found no skin penetration of fluorescein with less than 30 percent DMSO. DMSO may penetrate animal skin in concentrations above 40 percent (Moore and others 1970). It also has extremely low toxicity to humans, animals, and plants (Anon. 1984), and is exempted from tolerances on raw agricultural commodities when applied with certain pesticides (Federal Register 1983). DMSO toxicity is so low that it is measured in grams/kg (Wong and Reinertson 1984).

Summer is the least favorable time for SPB development, with RI in East Texas for 1960-62 remaining at less than 1:1 from June - October, and an average RI of 0.4 for June - July (Thatcher and Pickard 1964). Roton (1987) found that most associated insects were not affected by SMDC+DMSO treatment and a few increased significantly in treated trees. Our results also show that SMDC+DMSO infusion may supplement seasonal SPB mortality, especially during midsummer months. Observations of control and treated trees suggest that, though the RI during midsummer may be 0.42:1, SMDC+DMSO infusion lowers this by 10.5- to 14-fold (table 1). Therefore, induced resistance may be able to play a role in suppression of breakouts from summer cut-and-leave sites. If SMDC+DMSO infusion reduces RI in individual trees in infestations by a factor of 10, then collapse of treated spots would be expected. The treatment might also be of value from fall through spring, when ratios of increase are normally high.

This method does not rapidly reduce SPB. Studies on application methods are needed to improve consistency of results. The physiological mechanisms being stimulated and the necessary seasonal effects need to be identified to determine the biochemical basis for toxicity, the optimum time for application, and the greatest impact on beetle populations.

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Development of Analytical Methods for Escort Herbicide in Forest Environment Samples

Joseph B. Fischer and Jerry L. Michael

INTRODUCTION

The USDA Forest Service laboratory in Auburn, Alabama, is engaged in research to determine the environmental fate and ecosystem impacts of forestry herbicides. Much of the effort is associated with the conduct of field dissipation studies in which herbicides are applied to forest sites and then monitored over time in a variety of environmental matrices (soil, water, and plant tissue). While some methodology does exist for both sampling and analytical procedures, the available methods have often been developed in an ideal laboratory setting and are not directly suitable for forest environmental samples. Often, new methods must be developed to solve site-specific problems. This paper presents work completed to date on soil and water method development for a relatively new forestry herbicide, Escort.

Escort is the DuPont trade name for a formulation of metsulfuron methyl, a herbicide of the sulfonyl urea family, whose chemical name is methyl 2-[[[[(4-methyoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl] benzoic acid. Sulfonyl urea herbicides are much less stable chemically than other classes (like the phenoxy acids 2,4-D and 2,4,5-T) and break down rapidly following application. This makes them environmentally preferable, but it also presents challenges for storage and handling of field samples collected for environmental fate studies. Analytical methods for these herbicides must incorporate measures to minimize their decomposition between the time they are collected in the field and the time they are analyzed in the laboratory.

DEVELOPMENT OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) SEPARATION FOR METSULFURON METHYL

Metsulfuron methyl is similar chemically to sulfometuron methyl (trade name Oust), another sulfonyl urea herbicide previously studied in our laboratory. DuPont's published method for sulfometuron methyl (Zahnow 1985) utilized the Tracor 965 photoconductivity detector together with normal-phase HPLC on a bare silica column. We found this detector to be extremely temperamental and unsuitable for routine use. The hydrocarbon-based mobile phase proved to be incompatible with our Waters WISP autoinjectors, necessitating manual injections on round-the-clock shifts. The chromatographic system was itself unstable, requiring constant recalibration for changing retention times and responses,and periodic column rejuvenation whenever the system "crashed." We developed a more dependable autosampler-compatible reversed-phase HPLC separation for sulfometuron methyl using the ultraviolet absorbance detector, which proved highly successful (Wells and Michael 1987). These conditions were therefore used as the starting point for development of a metsulfuron methyl separation. Successive refinements of these conditions produced the following mobile phase/column combination, which is optimized for peak shape and retention time:

Column:	Supelco LC8-DB, 150 mm x 4.6 mm I.D., 5 um diameter spherical porous particles
Mobile phase:	30:70 (v/v) Acetonitrile/H_2O (0.1 M H_3PO_4, pH 2.0)
Flow rate:	1.0 ml/min at 25 °C
Detection:	U.V. Absorbance at 224 nm wavelength.

The optimum wavelength for detection was determined from the ultraviolet spectrum of metsulfuron methyl in the mobile phase. Under these conditions, metsulfuron methyl eluted at 6.5 minutes (fig. 1). The detection limit was 0.8 ng injected on-column, based on a minimum peak height five times the baseline noise level. Detector response was linear up to 2,000 ng injected.

DEVELOPMENT OF AN ANALYTICAL METHOD FOR METSULFURON METHYL IN WATER

Our method for analysis of metsulfuron methyl in water samples is derived from the method developed in our laboratory for sulfometuron methyl (Wells and Michael 1987). It is based on the principle of solid phase extraction (SPE). In SPE, a dilute solution of the analyte is passed through a column of adsorbent particles whose surface has a higher affinity for the analyte than does the solvent. The analyte is immobilized on the adsorbent, then released again by elution with a small volume of a more powerful solvent. In reversed-phase SPE, the analyte is nonpolar, the solvent is polar (usually water), and the adsorbent is nonpolar (usually silica gel derivatized with long-chain alkyl silane groups of up to 18 carbon atoms). Elution of the analyte is achieved with a less polar solvent, such as methanol.

Since metsulfuron methyl is a weak acid (pKa = 3.3), its ionization had to be suppressed in order to make it sufficiently nonpolar for reversed-phase SPE. This was accomplished by acidifying the water sample to pH 2.0 with 85 percent phosphoric acid. Elution from the SPE column was effected by acetonitrile or methanol, but the resulting concentrates were unstable, probably due to traces of phosphoric acid coeluted with the metsulfuron methyl. A better elution buffer was found to be 30:70 (v/v) acetonitrile/H_2O (pH 7.0/w 0.1 M potassium phosphate),

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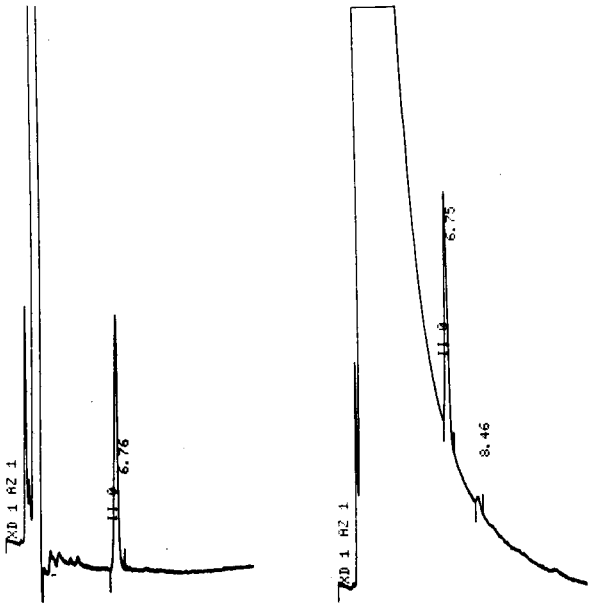

Metsulfuron Methyl Standard 20 ng injection

Spiked Well Water Extract 20 ppb Metsulfuron Methyl

Figure 1.--*LC chromatograms of metsulfuron methyl.*

which stabilized the extracts and was compatible with the HPLC mobile phase. Due to the extreme sensitivity reported for one aquatic species to metsulfuron methyl, the lowest possible detection limit was desired. This was done by maximizing the size of the water samples (to 500 ml) and minimizing the volume of the final eluted extract (to 5 ml).

Sulfonyl urea herbicides are known to be hydrolytically unstable in acid solution (E. I. Dupont de Nemours and Co., Inc. 1984). Samples of surface water taken from a treatment site in Florida had a pH value of 4.2. In order to determine the stability of metsulfuron methyl in field water samples of varying pH, a set of distilled water samples was buffered with 0.1 M potassium phosphate to pH 4.0, 5.0, and 6.0, then spiked with known amounts of metsulfuron methyl and stored at 35 °C. Five replicate samples at each pH level were analyzed every few days to monitor the percent age of the original spike remaining. The results are plotted in fig. 2. Hydrolysis was rapid at pH 4.0 and pH 5.0 and appreciable even at pH 6.0. It was therefore deemed necessary to add buffer to the sample containers in the field to stabilize the water samples as they were collected. Accordingly, a mixture of 0.04 M KH_2PO_4 and 0.06 M K_2HPO_4, which produces a pH of 7.0, was added in dry form to each 1 liter field container. The containers were frozen as soon as possible and kept frozen during transport and storage.

Since the water samples are adjusted to pH 2.0 to suppress their ionization prior to SPE, a recovery study was done to determine the effect of time at this pH on metsulfuron methyl recovery. A set of spiked water samples was adjusted to pH 2.0 and allowed to stand on the lab bench at 25 °C for periods of 1, 2, and 3 hours before being adsorbed on SPE columns and eluted with pH 7.0 buffer. Significant loss was observed even after 1 hour (fig. 3) reaching 15 percent by 3 hours. This loss was minimized by performing the SPE adsorption and elution steps as quickly as possible after acidification (less than 1 hour if possible) and by recording the time at pH 2.0 so that a correction factor could be applied if necessary. Prompt elution gave a recovery of 98.7 percent.

A test was conducted to determine if acidification to pH 2.0 was really necessary to get maximal adsorption of metsulfuron methyl on the C_{18}-SPE columns. A set of 500 ml water samples was buffered to pH 7.0 with 0.1 M potassium phosphate, then spiked with 40 ppb each of metsulfuron methyl. The pH of each spiked solution was then adjusted with 85 percent H_3PO_4 to values ranging from pH 2.0 to pH 7.0. These pH-adjusted water samples were then adsorbed and eluted from the SPE columns. Some of the columns were prewashed with methanol followed by pH 2.0 water and postwashed with pH 2.0 water. Some were prewashed with methanol and pH 2.0 water but not postwashed. Some were prewashed with methanol and pH

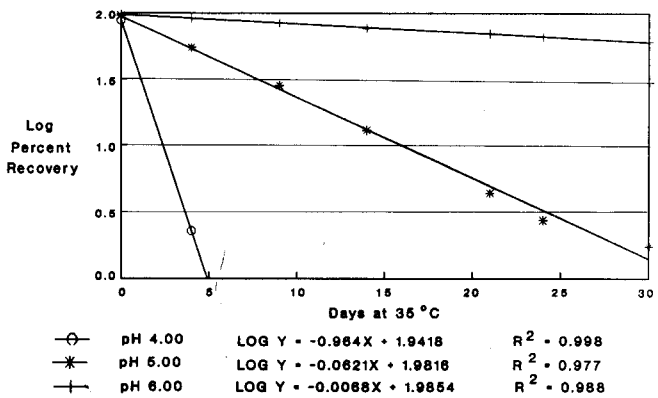

Figure 2.--*Effect of water sample pH on metsulfuron methyl recovery.*

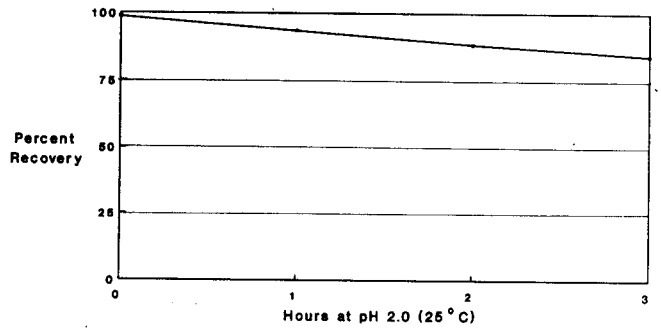

Figure 3.--*Effect of time at pH 2.0 on metsulfuron methyl recovery.*

7.0 buffered water with no postwash. The results are graphed in fig. 4. In all cases but one, the recoveries were in the range of 95-99 percent. Apparently, metsulfuron methyl is sufficiently insoluble in water that it adsorbs to the C_{18}-SPE columns even without ion-suppression by acid. The 0.1 M potassium phosphate buffer in the water samples may also contribute to suppression of ionization of metsulfuron methyl by the "salting-out" effect.

The final method is outlined as follows:

(1) Collect the water sample in the field from a well or an automatic streamflow sampler (ISCO), add 0.1 M potassium phosphate buffer to adjust the pH to 7.0, and freeze as soon as possible.

(2) Thaw the sample just prior to analysis. Measure the sample volume with a graduated cylinder.

(3) Precondition an SPE column containing 1000 mg of C_{18}-derivatized silica gel adsorbent (Baker #7020-7) by washing with 5 ml methanol followed by 10 ml of 0.1 M potassium phosphate buffer (pH 7.0).

(4) Pass the sample (500 ml) through an SPE column at a rate of 4 drops per second or less, controlled by adjusting the vacuum applied to the column.

(5) Elute the column with 4.5 ml 30:70 (v/v) acetonitrile/H_2O) (ph 7.0/w 0.1 M potassium phosphate buffer). Make up to exactly 5.0 ml with buffer. Mix thoroughly.

(6) Transfer the sample to an autosampler vial, filtering through a Millipore Millex-SR membrane filter if cloudy.

(7) Inject 10 μl on the HPLC. Quantitate against external standards injected before and after every fourth sample.

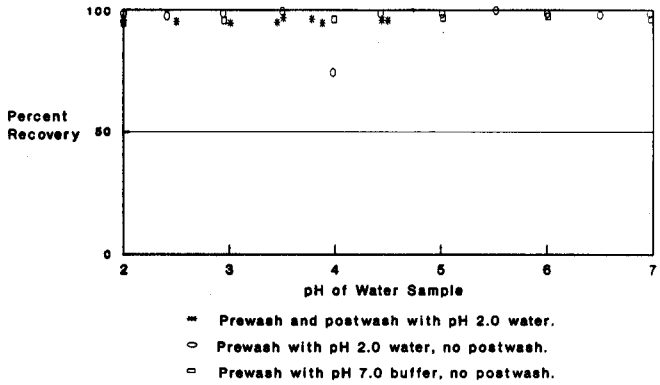

Figure 4.--*Metsulfuron methyl SPE recovery vs. pH of water extracted.*

Many samples of water from ponds and shallow wells at treatment sites are not clear, but instead tinted amber with dissolved organic matter. This complex polymeric material, principally humic and tannic acids, co-adsorbs and co-elutes with metsulfuron methyl from the solid phase adsorbent, competing for adsorption sites and reducing recovery. It also interferes with the HPLC separation, reducing the signal-to-noise ratio and lengthening equilibration time between injections. At the Florida treatment site, this has limited the overall concentration factor we can reliably use to 100:1 (500 ml water sample concentrated to 5 ml final extract) and the amount of extract we can safely inject on the HPLC system to 10 μl. This has made our practical detection limit in water about 1 ppb.

We have experimented with adding small amounts of divalent metal salts to "humified" water samples to precipitate the organic material prior to SPE. Lead (II) acetate (0.01 M) was very effective in precipitating organic material from unbuffered water, producing a clear supernatant and a flocculent brown precipitate that could be removed by suction filtration through glass fiber paper. Unfortunately, when this treatment was applied to water buffered with 0.1 M potassium phosphate, a grey precipitate of lead phosphate formed that interfered with filtration and left the organic material in solution. This treatment may prove more effective when applied to herbicides that do not require buffering for field stabilization.

DEVELOPMENT OF AN ANALYTICAL METHOD FOR METSULFURON METHYL IN SOILS

In environmental fate studies, it is necessary to sample soil at different depths to detect movement of the herbicide underground. In our laboratory, a method has evolved for collecting and processing soil samples that has proven effective for other herbicides (Michael and Neary 1988). A 2-inch I.D. PVC pipe is driven into the ground to a prescribed depth, then removed and frozen for transport to the laboratory. The frozen pipe is cut into 4-inch (10-cm) sections with a band saw. Each section is thawed, extruded, and spread out to dry on aluminum foil. When dry, each soil sample is screened to remove roots and pebbles, then refrozen in a zip-lock bag. The dry soil is passed through a 14-channel soil splitter several times until a representative sample of suitable size for analysis is obtained. The extraction of metsulfuron methyl proceeds as follows:

(1) A 50.0-g sample of the soil is weighed into a 250-ml, polypropylene, widemouth, screw-capped jar.

(2) A 100-ml portion of extracting buffer solution [50:50 (v/v) methanol/H_2O (pH 7.0/w 0.1 M potassium phosphate)] is added, and the mixture is shaken for 10 minutes on a table shaker.

(3) The mixture is centrifuged at 3,000 rpm for 10 minutes, and the supernatant is decanted.

(4) Steps 2 and 3 are repeated, and the supernatants are combined and suction-filtered through glass fiber paper (1 micron, GF/B, Whatman).

(5) The filtrate is rotary evaporated under reduced pressure on a warm water bath (45-50 °C) to remove the methanol component.

(6) The aqueous residue is extracted and eluted by SPE in the same manner as the water samples.

Unfortunately, polymeric materials also coextract with metsulfuron methyl from soils, and present serious problems in surface soils with high organic matter content. Soils low in organic matter give metsulfuron methyl recoveries of about 90 percent, whereas high organic soils can give recoveries as low as 70-80 percent. The detection limit is about 20 ppb for high organic soils.

Soils cannot be buffered as can water samples, and many forest soils have pH values around 4-5. We therefore ran an experiment to test the effect of soil drying time at room temperature on metsulfuron methyl recovery. A large sample of surface soil (pH 4.3) was tested to determine its field water capacity (43 percent w/w) and then was air-dried and subdivided into test samples using a soil splitter. These samples were individually treated with water to bring them to 25 percent, 50 percent, and 75 percent of their field capacity, then spiked with metsulfuron methyl and allowed to air-dry for 1, 2, and 4 days before being extracted and analyzed (fig. 4). We observed an appreciable drop in metsulfuron methyl recovery over the first 2 days of drying with a less rapid decline thereafter. This corresponds to visual observations that drying was complete by 2 days and suggests that hydrolysis is indeed taking place while the soil is damp. We are presently trying to modify our soil workup to eliminate the drying step and obtain a representative soil sample without splitting.

CONCLUSIONS

We have developed workable methods for analysis of metsulfuron methyl (Escort) herbicide in environmental samples of water and soil from forestry test sites. Detection limits are about 1 ppb in water and 20 ppb in soil. Measures for minimizing hydrolysis prior to analysis have been established for water and are under development for soil. Coextraction of dissolved organic matter remains a problem, especially for high organic soils.

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Evaluation of Worker Respiratory Exposure to Herbicide Residues in Prescribed Fire Smoke: A Preliminary Report

C. K. McMahon and P. B. Bush

INTRODUCTION

Occupational safety and health concerns have been raised in a number of southern States by workers conducting prescribed burns on forest lands treated with herbicides. Primary concern has focused on speculation that hazardous levels of herbicide residues are present in the smoke near breathing zones of forest workers. This speculation is fueled by fire hazard caution statements found on product labels and material safety data sheets. While these cautions are appropriate in connection with fires near herbicide storage sites, they were not intended to apply to fires following application on a forest site. In these cases, on a given acre, only a few ounces or pounds of active ingredient are spread over several tons of ground litter and forest vegetation, which becomes the dominant fuel for the prescribed fire.

Modeling assessments (Dost 1982, U. S. Department of Agriculture 1989) coupled with laboratory experiments (McMahon and others 1985, McMahon and Bush 1986) have shown that the herbicide risk to forest workers is insignificant, even if the fire occurs immediately after herbicide application. However, no field studies have been conducted to confirm these findings.

METHODS

To bridge that gap, a field validation study was conducted in Georgia in August-October 1988 to measure breathing zone concentrations of smoke particles, herbicide residues, and carbon monoxide (CO) on 14 operational site-preparation prescribed fires. Smoke was monitored on sites treated with labeled rates of imazapyr, triclopyr, hexazinone, and picloram (table 1) and burned operationally within 30-169 days after herbicide application. Tract size ranged from 6 to 380 acres (table 2). Preburn herbicide residues were estimated by analyzing samples of standing hardwood foliage, ground litter, and surface soil (table 3).

Personal monitors and area monitors employing glass fiber filters and polyurethane foam collection media were developed and validated for use in this study. A complete description of the monitors and analytical methods has been previously reported (McMahon and Bush 1990a, McMahon and Bush 1990b). The personal monitors were worn by forest workers to measure herbicide and "respirable" smoke particulate matter concentrations under a normal operational scenario. The area monitors were placed in zones of high smoke concentrations by research personnel to measure herbicide and "total" smoke particulate matter concentrations under a worst-case operational scenario.

RESULTS

No herbicide residues were detected in the smoke sampled from the 14 fires conducted in this study. This included a total of 48 personal monitors worn by forest workers, 22 personal monitors worn by research personnel, and 70 area monitors. The sensitivity of the monitoring methods were in the 0.1 to 4.0 $\mu g/m^3$ range, which is several hundred to several thousand times below any known herbicide inhalation risk level.

The monitoring of particulate matter and CO on these fires marks the first time worker exposure to these smoke constituents has been measured in the South. As expected, concentrations were highly variable depending on fire conditions and the location (upwind vs. downwind) of the personnel. Worker respirable (2.3 μm particle cutpoint) particulate matter concentrations ranged between 248 and 3,723 $\mu g/m^3$ with a mean of 1,429 $\mu g/m^3$. Exposure times depended on fire size and ranged from 1.2 to 6.3 hours with a mean of 2.8 hours (table 4). Area monitor total particulate concentrations ranged between 2,000 and 45,000 $\mu g/m^3$ with a mean of 8,311 $\mu g/m^3$. Based on the experiences of the research personnel who deployed these monitors, the area concentrations often exceeded tolerable long-duration working conditions. The respiratory distress and eye irritation associated with the higher values (above 8,000 $\mu g/m^3$) would prompt most workers to retreat to areas of lower smoke concentration after only a few minutes exposure. At this time, no occupational limits or guidelines have been established for worker exposure to either respirable or total smoke particulate matter.

Worker exposure to CO ranged from less than 6 to 23 ppm/hr while working on the fires. These values are well below the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for CO of 35 ppm/hr, when normalized to an 8-hour workshift.

In summary, the findings of this study support the "no significant herbicide risk" findings of the earlier modeling studies. In addition, the information on smoke particulate matter and carbon monoxide will be useful to those beginning to address other questions of worker safety and risk from exposure to forest fire smoke. These topics are discussed in more detail in the project final report (McMahon and Bush 1990b).

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Table 1.--*Herbicide formulations, active ingredients (ai), and common names of herbicides monitored in a smoke study in Georgia, August-October 1988*

Formulation	Active ingredient	Common name
Pronone 10G* Granular herbicide 10 percent ai soil active	hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl 1,3,5-triazine-2,4(1H, 3H)-dione)	Hexazinone
Velpar ULW† Granular herbicide 75 percent ai soil active	hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H, 3H)-dione)	Hexazinone
Garlon 4‡ Liquid herbicide 61.6 percent ai 44.3 percent ae§ foliar active	Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), butoxyethyl ester	Triclopyr
Arsenal†† Applicators Concentrate Liquid herbicide 53.1 percent ai 43.3 percent ae foliar and soil active	Imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridine carboxylic acid), isopropyl-amine salt	Imazapyr
Tordon K‡‡ Liquid herbicide 24.0 percent ai 20.8 percent ae foliar and soil active	Picloram (4-amino-3,5,6-trichloropiclinic acid) potassium salt	Picloram

* Trademark, Pro-Serv Inc.
† Trademark, E.I. duPont de Nemours and Company, Inc.
‡ Trademark, DowElanco
§ ae=acid equivalent
†† Trademark, American Cyanamid Company
‡‡ Trademark, DowElanco

Table 2.--*Prescribed fire study areas and treatments in 1988*

Fire no.	Location	Acres burned	Herbicide treatment formulation and rate	Date of herbicide application (A)	Burn date (B)	Interval (days) (B-A)	Herbicide ai* monitored in smoke
1	Bainbridge, GA	42	Pronone 10G, 25 lb/ac	5/20/88	8/29/88	101	Hexazinone
2	Bainbridge, GA	7	Garlon 4, 3.5 qts/ac	7/29/88	8/30/88	32	Triclopyr
3	Bainbridge, GA	13	Accord, 2 qt/ac; Garlon 4, 2 qt/ac	7/29/88	8/30/88	32	Triclopyr
4	Bainbridge, GA	8	Accord, 5 qt/ac	7/8/88	9/13/88	67	None
5	Bainbridge, GA	42	Arsenal†, 1 pt/ac; Accord, 3 pt/ac	7/7/88	9/14/88	69	Imazapyr
6	Bainbridge, GA	32	Arsenal, 1 pt/ac; Garlon 4, 3 pt/ac	8/2/88	9/15/88	44	Triclopyr
7	Harris Cty, GA	200	Velpar ULW, 4.7 lb/ac	4/13/88	9/27/88	167	Hexazinone
8	Harris Cty, GA	380	Velpar ULW, 4.7 lb/ac	4/13/88	9/28/88	168	Hexazinone
9	Meriweather Cty, GA	320	Velpar ULW, 4.7 lb/ac	4/13/88	9/29/88	169	Hexazinone
10	Bainbridge, GA	6	Arsenal 3 pt/ac‡	9/25/88	10/25/88	30	Imazypyr
11	Bainbridge, GA	42	Pronone 10G, 25 lb/ac	5/20/88	10/25/88	158	Hexazinone
12(A)	Bainbridge, GA	48§	Arsenal, 3 pt/ac‡	7/12/88	10/26/88	106	Imazypyr
12(B)	Bainbridge, GA		Arsenal, 1.5 pt/ac	7/12/88	10/26/88	106	Imazypyr
12(C)	Bainbridge, GA		Arsenal, 1 pt/ac	7/12/88	10/26/88	106	Imazypyr
13(A)	Bainbridge, GA	48††	Garlon 4, 2 qt/ac, Tordon K, 2 qt/ac	7/21/88	10/26/88	97	Triclopyr & Picloram
13(B)	Bainbridge, GA		Garlon 4, 2 qt/ac, Tordon K, 2 qt/ac	7/21/88	10/26/88	97	Triclopyr & Picloram
14	Bainbridge, GA	6	No herbicides applied	---	10/27/88	--	None

* ai = active ingredient. Except for triclopyr and picloram, smoke monitoring procedures only allowed sampling for one herbicide when tank mixtures were used. Glyphosate (Accord) was not included in the monitoring program for this study.

† All present treatments were with Arsenal Applicators Concentrate formulation.

‡ Three-pint rate applied under an experimental use permit; for research purposes, on sites 10 acres or less. Under normal use, the maximum label rate for Arsenal Applicators Concentrate is 2.5 pints/acre.

§ Three sub-plots, 10, 15, and 23 acres respectively.

†† Two sub-plots, 24 acres each.

Table 3.--*Herbicide residues in preburn site samples**

		Hardwood foliage	Ground litter	Surface soil
a. Hexazinone residues				
Fire #1	101 DAT†	0.08 ± 0.50	0.06 ± 0.04	0.24 ± 0.20
Fire #7 & #8	167 DAT	<0.05	<0.05	0.58 ± 0.54
Fire #9	169 DAT	0.12 ± 0.11	<0.05	0.25 ± 0.21
b. Imazypyr residues				
Fire #5	69 DAT	<0.5	<0.5	<0.5
Fire #10	1 DAT ‡	4.6 ± 3.2	4.1 ± 4.0	<0.5
Fire #10	30 DAT	1.5 ± 1.2	2.1 ± 2.6	<0.5
Fire #12ABC	106 DAT	<0.5	<0.5	<0.5
c. Triclopyr residues				
Fire #2	32 DAT	21.0 ± 23.7	11.2 ± 10.1	0.16 ± 0.11
Fire #3	32 DAT	9.9 ± 9.8	4.3 ± 2.3	0.43 ± 0.60
Fire #6	44 DAT	10.6 ± 5.9	2.8 ± 1.7	0.21 ± 0.29
Fire #13AB	97 DAT	8.4 ± 13.2	1.6 ± 1.1	0.54 ± 0.43
d. Picloram residues				
Fire #13AB	97 DAT	1.14 ± 1.19	0.35 ± 0.28	0.21 ± 0.22

* Values in mg/kg. Average ± standard deviation, n=6. Detection limits for hexazinone, triclopyr, and picloram, 0.05 mg/kg; for imazapyr 0.5 mg/kg. In those cases where a sample was below the detection level, one-half the detection level was used in computing the average value.

† DAT = Days after herbicide treatment.

‡ Fire # 10 burned 30 DAT, however, site samples were also taken 1 DAT.

Table 4.--*Smoke respirable particulate matter concentrations and exposure times for forest workers (by fire size)*

	Particulate matter ($\mu g/m^3$)			Exposure time Hours ± S.D.
	Low	High	Mean ± S.D.	
Small fires (8 ac av) Fires 2,3,4,10,14	405	2,312	1,118 ± 625	1.4 ± 0.2
Medium fires (42 ac av) Fires 1,5,6,11,12,13	694	2,375	1,573 ± 498	2.3 ± 0.4
Large fires (300 ac av) Fires 7,8,9	248	3,723	1,596 ± 1,140	4.7 ± 0.8
All fires	248	3,723	1,429 ± 801	2.8 ± 1.8

*Particle 50 percent cutpoint, 2.3 um (aerodynamic diameter).

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NMR Experiments for Elucidation of the Structure of Flavanoid Oligomers: Some Examples

Richard W. Hemingway, Jan P. Steynberg, and Seiji Ohara

INTRODUCTION

The phenomenal advance in our understanding of the chemistry of condensed tannins and related flavanoid derivatives over the past 10 years is largely due to new NMR experiments made possible by modern superconducting FT spectrometers. For example, it was only 10 years ago, in the Alexandria Forestry Center, coincident with Lawrence Porter's work in New Zealand, that ^{13}C-NMR spectra of condensed tannin polymers extracted from conifer tree barks were recorded (Karchesy and Hemingway 1980, Czochanska and others 1980). These spectra contain a vast quantity of information about the structure of flavanoid polymers (1) (fig. 1) including the hydroxylation pattern of the A- and B-rings, the stereochemistry of the chain extender units, the stereochemistry of the terminal units, and an approximation of the number average molecular weight of the polymers (fig. 1) (Porter and others 1982, Newman and others 1982). However, new two-dimensional experiments are so powerful that few studies of flavanoid oligomers or their derivatives are now based on a simple ^{1}H- or proton decoupled ^{13}C-NMR experiments. The objective of this report is to present some examples of how modern NMR experiments have been used to determine the structure of flavanoid oligomers in hopes that they might be of use to others addressing structural problems in the Southern Station.

RESULTS AND DISCUSSION

^{13}C-NMR and the Stereochemistry of the Heterocyclic Ring in Profisetinidins. Although ^{13}C-NMR has been widely used in the study of the procyanidins (polymers based on 3,5,7,3',4'-pentahydroxyflavans; i.e. (1)), it has been largely ignored in study of the profisetindins (polymers based on 3,7,3',4'-tetrahydroxyflavans). Jan Steynberg has made a series of profisetinidins during his studies in Pineville (fig. 2) (Steynberg and others, in preparation-b). These compounds have made possible a study of the influence of stereochemistry on the chemical shifts of carbons in the heterocyclic rings of these compounds (Steynberg and others, in preparation-a). Even a dimeric compound contains five chiral centers, so definition of the relative stereochemistry by NMR methods is very important. The absolute stereochemistry can then normally be defined from CD spectra, which establish the absolute stereochemistry at the C-4 position:

The relative stereochemistry at C-2 and C-3 is readily apparent in ^{13}C-NMR spectra because of the large downfield shift of the C-2 carbon signal in 2,3-*trans* isomers such as (2) or (3) figure 2, in comparison to the chemical shift of C-2 in the 2,3-*cis* isomers such as (4) or (5) (See fig. 3-5). In contrast to the ^{13}C-NMR spectra of procyanidins (1), spectra of the 2,3-*trans* profisetinidins such as (2) or (3) also reveal the relative stereochemistry at C-4 by the large downfield shift of the C-4 signal in the 2,3-*trans*-3,4-*cis* isomers. Definition of the stereochemistry of the terminal units is also straightforward from the ^{13}C-NMR spectra (fig. 5) where the 2,3-*trans* isomers show resonances for C-2 and C-3 at about 82 and 68 ppm, whereas these carbons of 2,3-*cis* terminal units appear at about 79 and 66 ppm, respectively. One of the primary advantages of ^{13}C-NMR is that plant extracts containing proanthocyanidins can be easily surveyed for hydroxylation pattern and stereochemistry by ^{13}C-NMR without the necessity of making or isolating individual compounds. Another advantage is that we can learn something about the conformational properties of these compounds in the free phenolic state. The spectrum of the 2,3-*trans*-3,4-*trans* isomer (2) is a case in point (fig. 3). Doubling of the C-2 carbon resonance is considered to be due to two rotational isomers, and integration of these signals provides an estimate of the relative proportions of each. We can also see doubling of the C-4 carbon of the C-ring. Because of symmetry (fig. 6) with respect to this carbon in the two rotamers, we might attribute doubling of this carbon signal to differences in the proportions of A and E conformers between the two rotamers (fig. 7).

^{1}H n.O.e. Difference Spectra for Proof of the Location of Substitution and evidence for Rotational Isomerism in a Trimer of Mixed Stereochemistry. The great power of ^{1}H n.O.e. difference experiments is demonstrated in its application to determine the structure of a trimer of mixed stereochemistry that was isolated from Guamuchil bark (Steynberg and others, in preparation-a). A ^{1}H-nmr spectra showed that this trimer contained both a 2,3-*trans*-3,4-*trans* fisetinidiol unit and a 2,3-*cis*-3,4-*cis* *ent*-epifisetinidol unit substituted at either C-6 or C-8 of a terminal 2,3-*cis* epicatechin unit [i.e., (6)]. Assignment of the location of substitution was possible from consideration of n.O.e. enhancements from the 5-OMe and 7-OMe of the D-ring (fig. 8). Since only the 7-OMe would result in enhancement of the 4-H of both rings C and I, the 2,3-*trans*-3,4-*trans* unit with $J_{2,3}$ and $J_{3,4}$ both about 10 Hz must be the unit at C-8 and the 2,3-*cis*-3,4-*cis* unit with coupling constants of $J_{2,3}$ = 3.0 and $J_{3,4}$ = 5.0 Hz must be at C-6 of the D-ring.

This same experiment also provides evidence on the rotational mobility of the two profisetinidin units in this trimer. The 2,3-*cis*-3,4-*cis* unit substituted at C-6 of ring D rotates freely about the interflavanoid bond as evidenced by the n.O.e. enhancement of the 5-$H_{(G)}$ proton from both 5-

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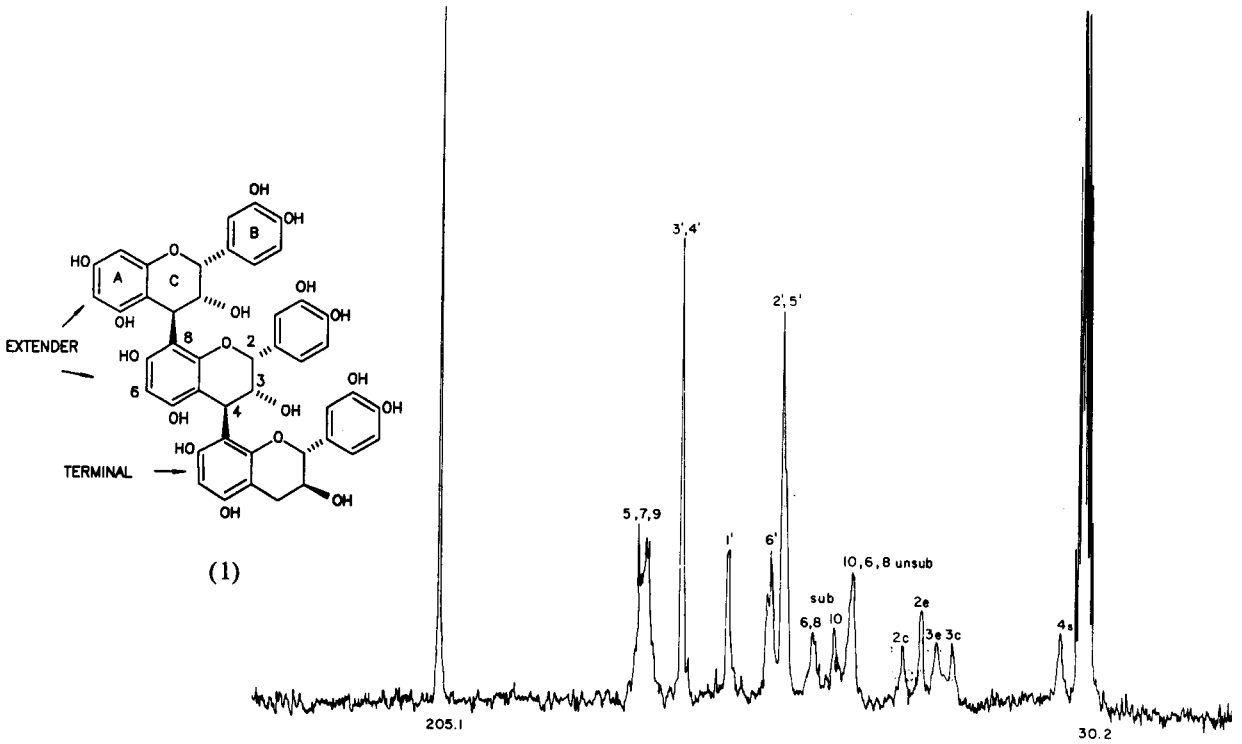

Figure 1.--*The first ^{13}C-NMR spectrum recorded for a tannin extract from a conifer tree bark.*

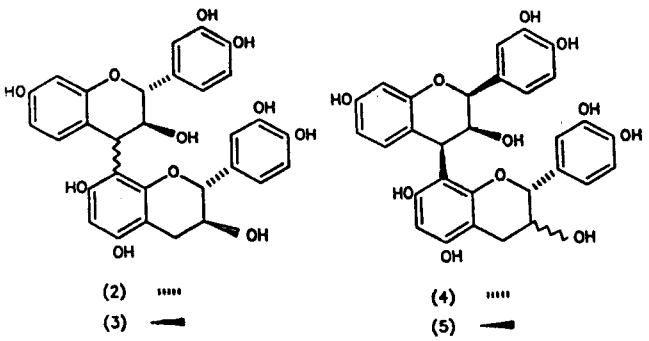

Figure 2.--*Structures of profisetinidins illustrating variations in stereochemistry.*

$OMe_{(D)}$ and 7-$OMe_{(D)}$. For this to occur, there must be comparatively free rotation about this bond. In contrast, the 2,3-*trans*-3,4-*trans* unit at C-8 of the D-ring does not appear to rotate freely as suggested from the absence of an n.O.e. enhancement between the 7-$OMe_{(D)}$ and the 5-$H_{(A)}$. The freedom of rotation observed for methyl ether acetate derivatives of the 2,3-*cis*-3,4-*cis* profisetinidin units is most unusual.

C-H HETCORR Experiment to Assign Carbon Signals in the Propyl Chain and Nonatrione Ring System of a Diarylpropanol-Catechinic Acid Dimer. During Seiji Ohara's year of study in Pineville, he made an interesting compound **(7)** that suggests stereospecific intra- and intermolecular reactions of catechin in base (fig. 9) (Ohara and Hemingway in press). There are many instances in which it is difficult if not impossible to assign carbon signals when these spectra are considered alone. The complexity of the proton decoupled ^{13}C-NMR spectrum of the methyl ether derivative of Ohara's compound **(8)** is a good example (fig. 10). However, the Hetcorr experiment (fig. 11) solves most of these problems. This connectivity experiment allows us to determine what protons are attached to what carbons. By combining information on the chemical shifts and coupling constants obtained from the proton spectra with chemical shifts of the attached carbons one can usually assign the spectra and solve the structure. For example, connectivity between the propyl 1-H at 4.06 with the carbon signal at 49.63 and the 2-H at 5.20 with the carbon signal shifted far downfield at 94.71 is clear from this spectrum. The C-3 carbon is assigned to the signal at 28.1 by its connectivity with the double doublets centered at 2.90 and 3.10 ppm. In addition, most of the carbons of the nonatrione ring C system are readily assigned from consideration of the Hetcorr experiment. The proton signal at 3.32 ppm assigned to 1-H was correlated with the carbon signal at 66.63, and the

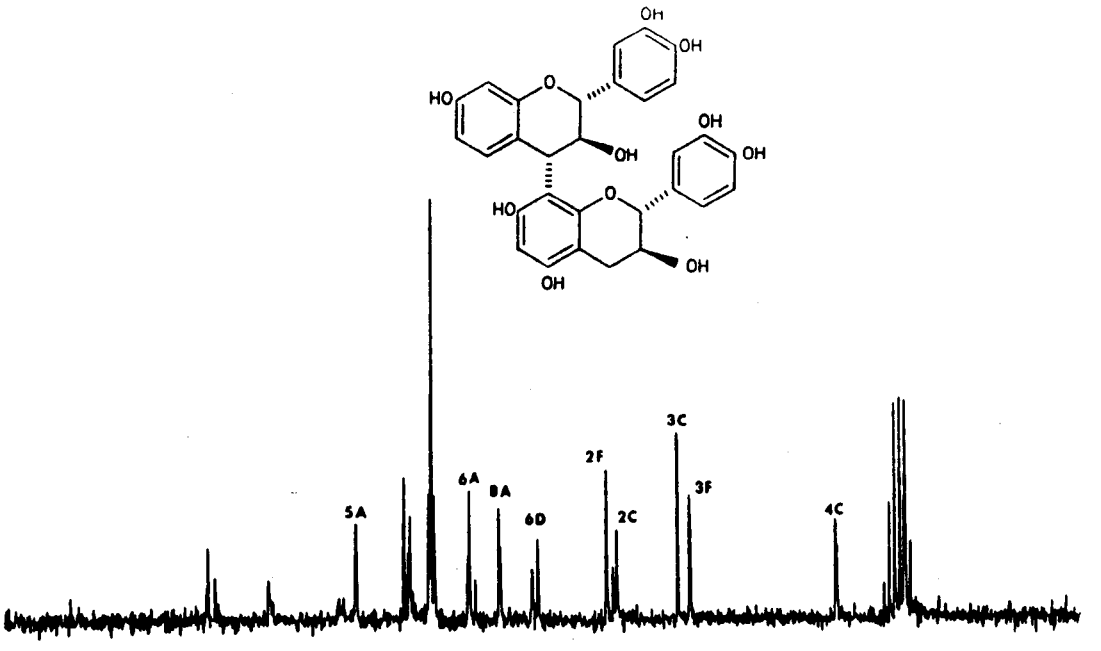

Figure 3.--^{13}C-NMR *spectrum of 2,3-trans-3,4-trans* (**2**).

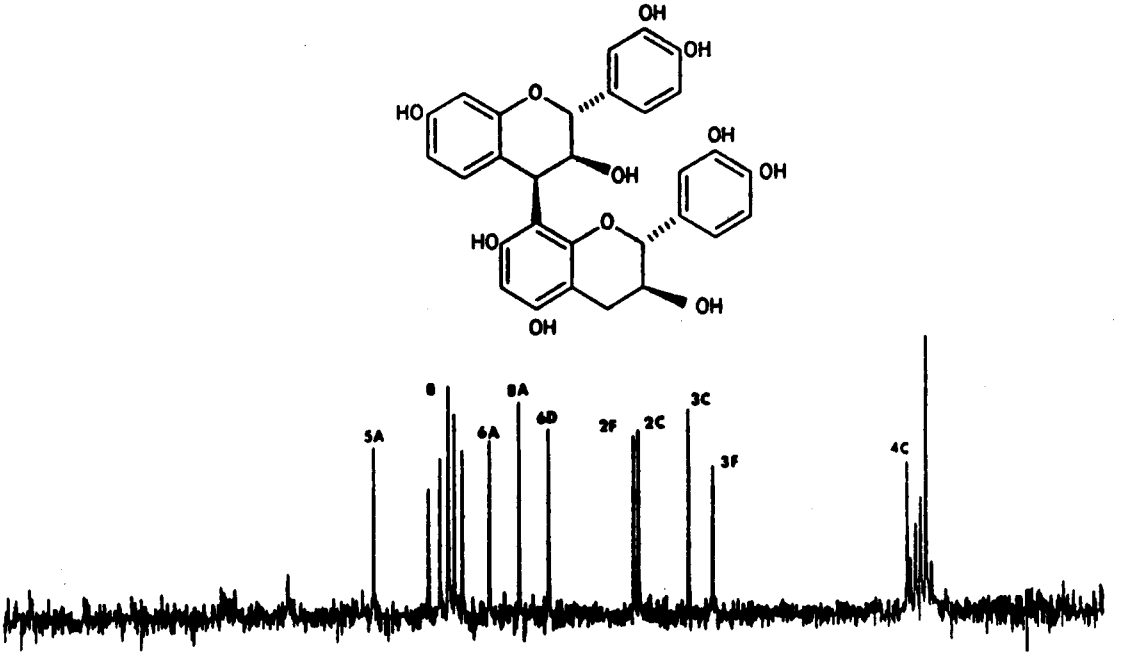

Figure 4.--^{13}C-NMR *spectrum of 2,3-trans-3,4-cis* (**3**).

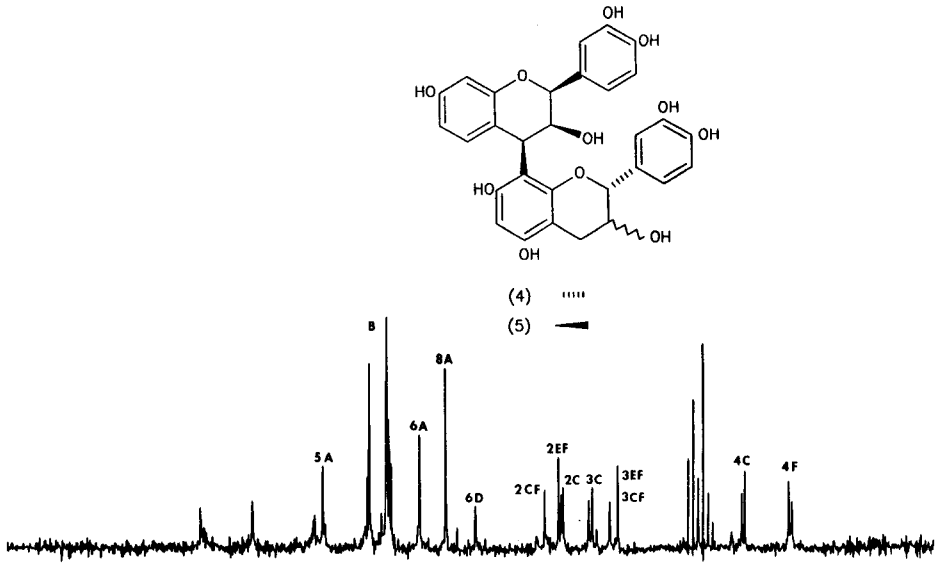

Figure 5.--*^{13}C-NMR spectrum of a mixture of 2,3-*cis*-3,4-*cis* with epicatechin and catechin terminal units* (**4**) *or* (**5**).

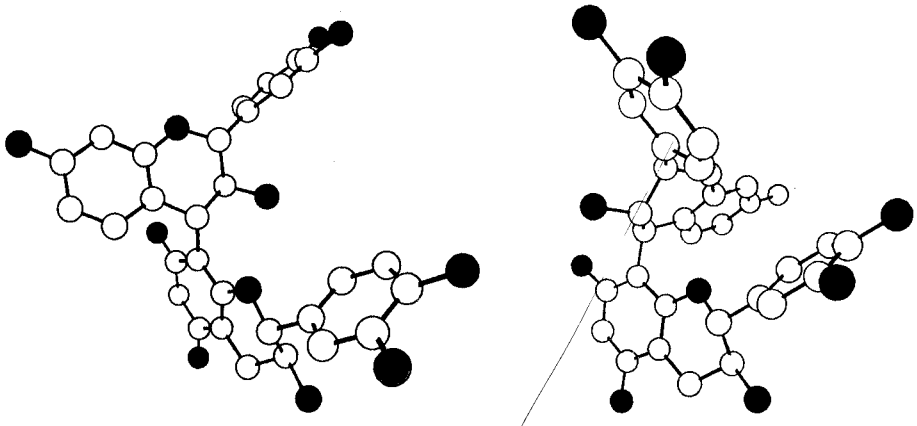

Figure 6.--*Two rotameric forms of a profisetinidin.*

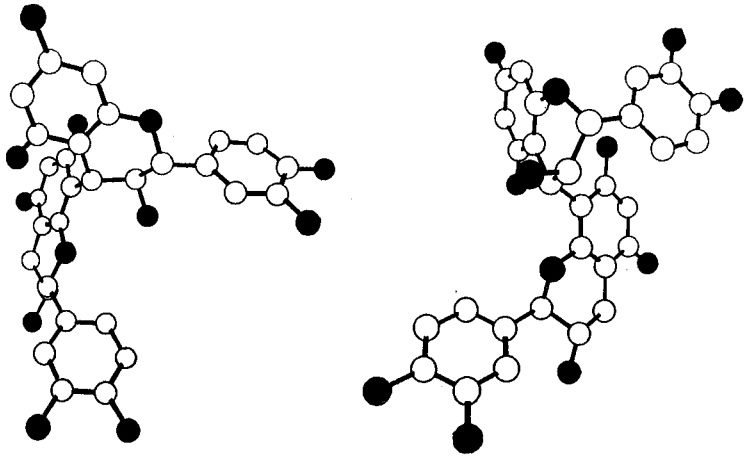

Figure 7.--*A and E conformers of a procyanidin, catechin-(4a --> 8)-catechin.*

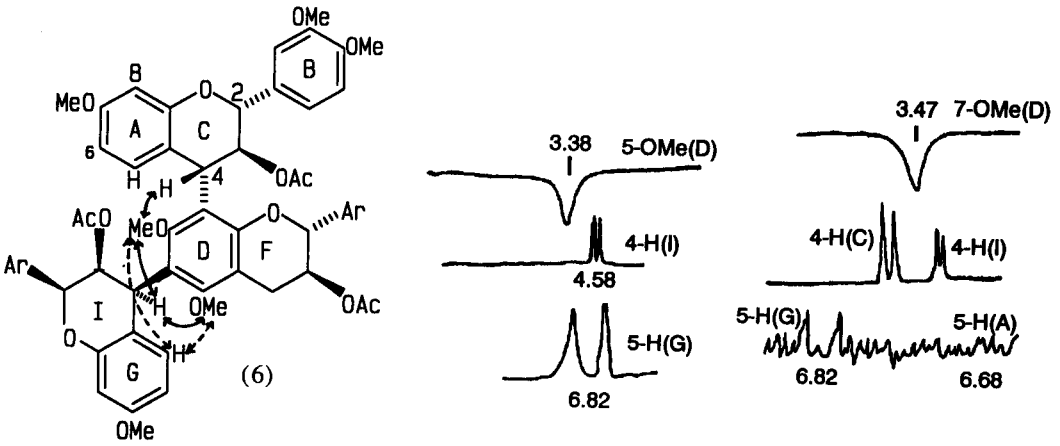

Figure 8.--*An n.O.e difference experiment showing the location of substitution of 2,3-*trans *and 2,3-*cis *profisetinidin units on a trimer.*

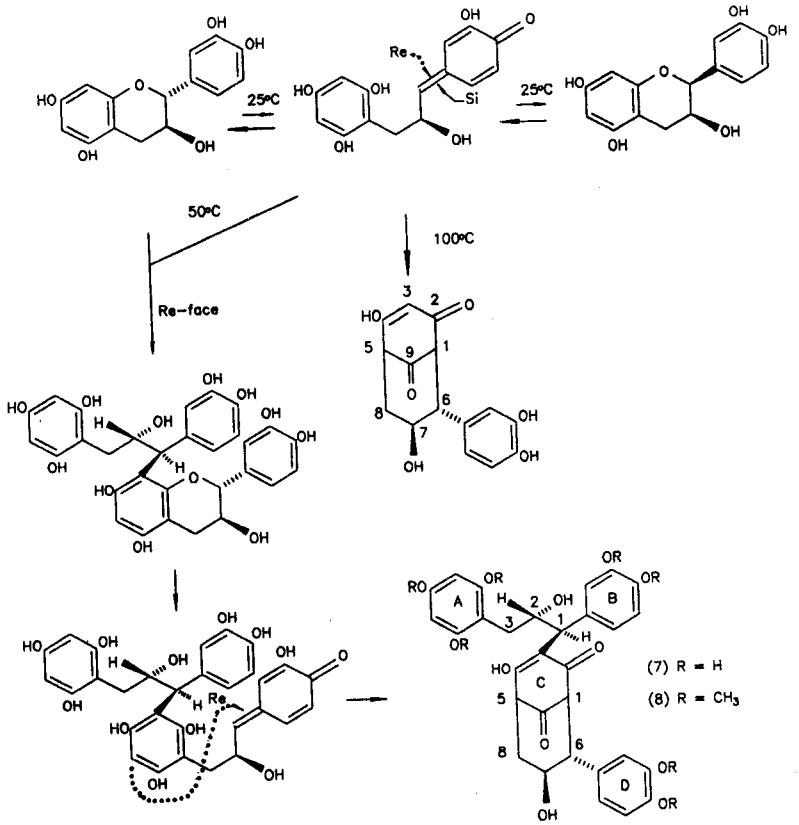

Figure 9.--*Stereoselective base-catalyzed reactions of (+)-catechin.*

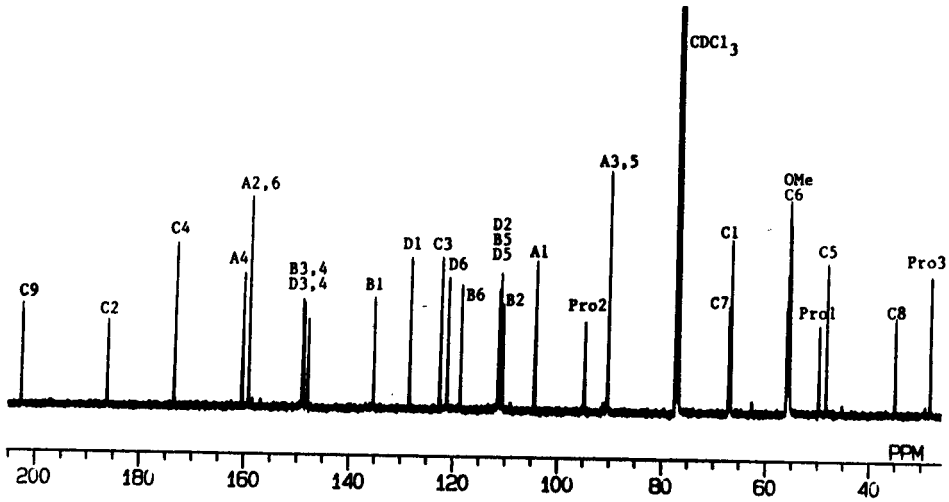

Figure 10.--*^{13}C-NMR spectrum of the methyl ether derivative of the main reaction product of base-catalyzed reactions of catechin at 40 °C.*

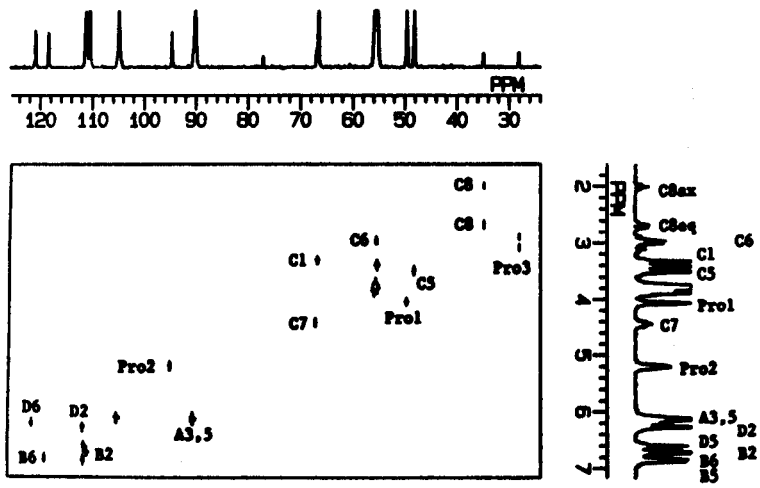

Figure 11.--*The ^{13}C-^{1}H Hetcorr spectrum of (8).*

multiplet at ppm that is clearly 7-H is correlated with the close signal at 67.03. The broad signal at 3.50 ppm assigned to 5-H is correlated with the carbon signal at 48.2. Similar correlations for the 6 and 8 carbons permitted assignment of all carbon signals except the carbonyls.

CONCLUSIONS

These examples are but a few of the experiments that are required to obtain proof of the structure of flavanoid oligomers by NMR methods. Other ^{1}H n.O.e. difference experiments are useful for assignment of the conformational features of these compounds and are described more fully by Ferreira and Brandt (1989). A recent book (Martin and Zektzer 1988) provides very readable additional details on the nature of these experiments and their application to the elucidation of structure of complex organic compounds.

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Steynberg, J.P.; Ohara, S.; Hemingway, R.W. [and others]. [In preparation]b. Condensed tannins: isolation of 2S,3S-profesetinidins from Guamuchil, and observations on their synthesis and biogenesis.

Principles and Applications of Relaxation Spectroscopy

Timothy G. Rials

INTRODUCTION

Amorphous polymers can readily be classified into two distinct groups according to their mechanical behavior. Some polymers like polystyrene and poly(methyl methacrylate) are rigid, *glassy* plastics at room temperature, whereas others are flexible, *rubbery* materials such as polybutadiene and polyisoprene. However, if polystyrene is heated to about 125 °C, it exhibits typical rubbery behavior, and when polybutadiene is cooled in liquid nitrogen, it becomes rigid and glassy. The temperature at which this change in properties occurs is known as the glass transition temperature, T_g, a primary relaxation process in polymers, and involves large-scale molecular motion (Seymour and Carraher 1988, Rosen 1987). Consequently, whether a polymer exhibits rubbery or glassy properties depends on the relative position of its application temperature to its T_g.

This primary relaxation process ultimately determines the service conditions of a given polymer, but these materials typically exhibit at least one other secondary relaxation process below T_g. Glassy state relaxations are attributed to motions of groups of atoms smaller than those necessary to produce a glass- to-rubber transition and can significantly influence mechanical properties. As an example, polycarbonate (bullet-proof glass) exhibits a strong relaxation process below room temperature that is not present in brittle plastics like polystyrene. In addition, relaxation processes may arise as a consequence of multiple phases (e.g., crystallinity) or multiple components (e.g., polymer blends, copolymers, fiber composites), providing an approach to modifying the mechanical properties of a material. Therefore, interpretation of relaxation properties at the molecular level is of great scientific and practical importance in understanding the mechanical behavior of polymers. Several excellent reviews of relaxation properties in relation to the molecular structure of polymers are available (Aklonis and MacKnight 1983, Nielsen 1974, Ferry 1980, Murayama 1987).

The study of molecular motion in polymeric materials has been led by nuclear magnetic resonance spectroscopy; however, recent years have seen the appearance of several new analytical techniques including dynamic mechanical analysis and, more recently, dielectric analysis. The recent acquisition of these two instruments at the Forest Products Utilization Research Laboratory has greatly expanded the polymer characterization capability in the Southern Station. A brief introduction to these new techniques in terms of their principles of operation, as well as exemplary applications, is presented in the following discussion.

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FUNDAMENTALS AND INTERPRETATION

Dynamic relaxation experiments rely on the fundamentally different response of a spring and a dashpot to a sinusoidally applied stress (Aklonis and MacKnight 1983). The response of an ideally elastic material (the spring) is exactly inphase with the applied stress, whereas a purely viscous material (the dashpot) lags behind by a 90° phase angle. In the more realistic case of a viscoelastic material, the response lags behind by some intermediate phase angle, δ, since a portion of the energy associated with that stress is stored through an elastic response, whereas another portion is lost due to molecular friction. From this information, the complex response can then be resolved into its individual components. For example, from the sinusoidal mechanical wave applied in dynamic mechanical analysis, the parameters derived include the storage modulus (E'), the loss modulus (E"), and the dissipation factor (tan δ). To a first approximation, dielectric analysis presents an analogous situation with the exception that an electrical force, rather than a mechanical deformation, is applied. The input and output for these methods are compared in table 1.

Table 1.--*Comparison of dynamic mechanical and dielectric analysis*

Parameter	Dynamic mechanical thermal analysis	Dielectric thermal analysis
Input	Stress	Voltage
Output	Strain	Current
Properties		
Elastic component	Storage modulus (E')	Dielectric const. (ϵ')
Viscous component	Loss modulus (E'')	Dielectric loss (ϵ'')
Other	Tan delta (E''/E')	Tan delta (ϵ''/ϵ')
		Ionic conductivity

In general, relaxation spectroscopy focuses on defining the variation in the individual response mechanisms with temperature, as illustrated through the following example. Figure 1 shows the variation in the dynamic mechanical properties, Log E' and tan δ, for poly(methyl methacrylate), a typical amorphous polymer, over the temperature range -50 °C to 160 °C. At low temperatures, a modulus of about 10^{10} dynes/cm^2 is observed, which is typical of a glassy polymer. A precipitous drop in the modulus to about 10^6 dynes/cm^2 is encountered around 110 °C to 130 °C along with a sharp peak in tan δ. This relaxation process corresponds to the T_g of PMMA and is indicative of large-scale molecular motion. An additional relaxation process is centered around 50 °C in the tan δ spectrum, but is accompanied by a much smaller decline in modulus. This secondary relaxation, termed the ß relaxation, arises from the motion of the $-CO_2CH_3$ side groups. It is important to note that the time scale of the experiment can influence the relaxation behavior in a manner

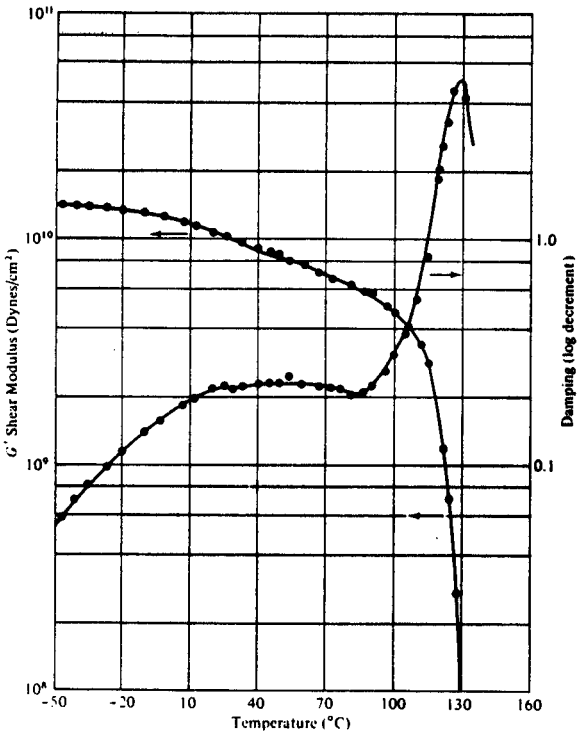

Figure 1.--*The effect of temperature on the dynamic mechanical properties (Log E' and tan δ) of poly(methyl methacrylate).*

similar to temperature. That is, a viscoelastic material will become more glasslike as the frequency is increased and the time-dependent response mechanisms are unable to respond in time. Consequently, the typical glassy modulus of the polymer may be observed even at temperatures well above its glass transition temperature. The complete characterization of a material's relaxation properties must, then, include both time and temperature as variables.

As mentioned earlier, dielectric relaxation spectroscopy is similar in principle to dynamic mechanical analysis; however, it is phenomenologically distinctive (Aklonis and MacKnight 1983, Hedvig 1977). This technique is concerned with the time/temperature dependence of the dielectric constant and, therefore, focuses on dipolar (e.g., hydroxyl or carbonyl) relaxation. Dielectric analysis provides a wider frequency range and eliminates sample restrictions, making it an extremely versatile, stand-alone, analytical tool (Day 1988, Grentzer and Leckenby 1989). In addition, dielectric analysis is often more sensitive to glassy state relaxations and, therefore, provides a complementary technique to dynamic mechanical experiments. Because of the sensitivity of these methods to a number of different types of molecular motions, transitions, and relaxation processes, dynamic mechanical and dielectric analyses find applications in a wide variety of polymeric systems.

APPLICATIONS OF RELAXATION SPECTROSCOPY

The Wood Composite. Because of the complexity of wood at the macroscopic level, it is convenient to overlook the fact that the woody cell wall is itself a unique composite consisting of three structural polymers. The system is generally described as a fibrous, cellulosic component embedded in an amorphous matrix of hemicellulose and lignin (Erins and others 1976); however, very little information is available on the *in situ* morphology (i.e., the extent of molecular mixing) of the blend of polymers comprising the amorphous element of the cell wall. Dynamic mechanical analysis has recently been used to address this question by evaluating the influence of moisture on the viscoelastic response of wood (Kelley and others 1987).

The presence of a low molecular weight species often serves to "plasticize" an amorphous polymer, lowering the energy requirements of chain mobility (Rudin 1982). Consequently, the precise temperature at which the primary dispersion occurs can be expected to vary with moisture content as is illustrated in figure 2. At low moisture content, the glass transition temperature of lignin (α_1) and hemicellulose (α_2) are barely detectable, well above 100 °C, and are indistinguishable from each other. The plasticizing efficiency of water, however, varies considerably for the two relaxations. Whereas the lignin transition declines to 60 °C as moisture content rises to 10 percent and remains constant, the hemicellulose transition continues to decline to subambient temperatures with increasing moisture content.

The resolution of two separate T_g values confirms the immiscibility of the two amorphous wood polymers, lignin and hemicellulose. Of particular interest is the observation that hemicellulose exhibits a subambient glass transition at relatively low moisture contents, whereas lignin remains glassy even at high moisture contents. The combined rubbery and glassy response may contribute to the overall toughness of the composite system in a manner similar to rubber-toughened epoxies. In light of the importance of moisture on the processing of wood and pulp fibers, the recognition of this viscoelastic response may have significant implications in the production of wood composites.

Wood Fiber/Polymer Composites. The utilization of wood as a reinforcing fiber in thermoplastic polymers has recently received considerable attention (Takase and Shiraishi 1989, Dalvag and others 1985, Woodhams and others 1984). The strength and toughness of this natural fiber offer a number of advantages over conventional reinforcements, but present the problem of limited compatibility with commodity thermoplastics such as polyethylene or polypropylene. The incompatible nature of the two components results in an inadequate interfacial region that prohibits the successful transfer of stresses to the load-bearing fiber. Thus, much of the research effort in this area has focused on fiber modification reactions aimed at enhancing the quality of the fiber/polymer interface. Dynamic mechanical analysis provides a convenient technique to study the extent of

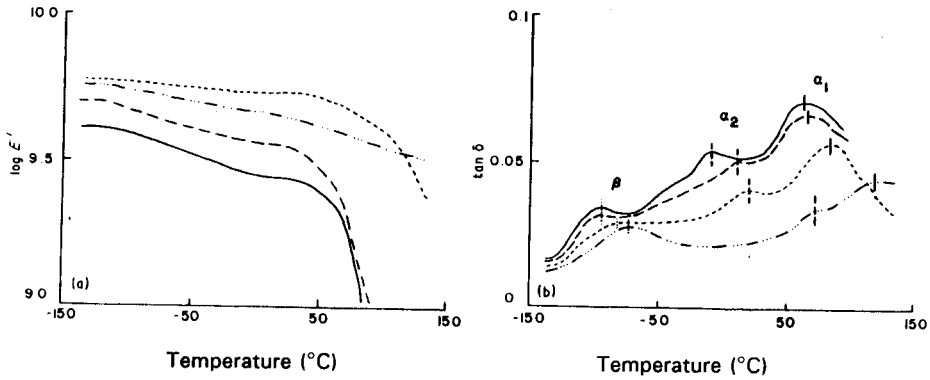

Figure 2.--*The influence of moisture on the dynamic mechanical response (a) log E' and (b) tan δ of spruce wood. Moisture contents are: 5 percent (_._), 10 percent (---), 20 percent (_ _), and 30 percent (__).*

interaction between the individual components of the material and has recently been applied to wood fiber/polypropylene composites (Krebaum and others 1990).

Figure 3 shows the variation in the storage modulus (E') and tan δ with temperature for several polypropylene composites containing 50 wt. percent wood fiber. The materials were obtained by disk-refining red oak chips and include the control fiber, a common titanate coupling agent modification, and modification with a long chain fatty amine. There are two events common to all three spectra, the most notable being the melting transition of polypropylene (a semicrystalline polymer) at high temperature (Quirk and Alsamarraie 1989). Interestingly, the melting temperature (T_m) is depressed from 158 °C for the titanate-modified fiber to 150 °C for the fatty amine modification and the unmodified fiber composites, indicating more favorable

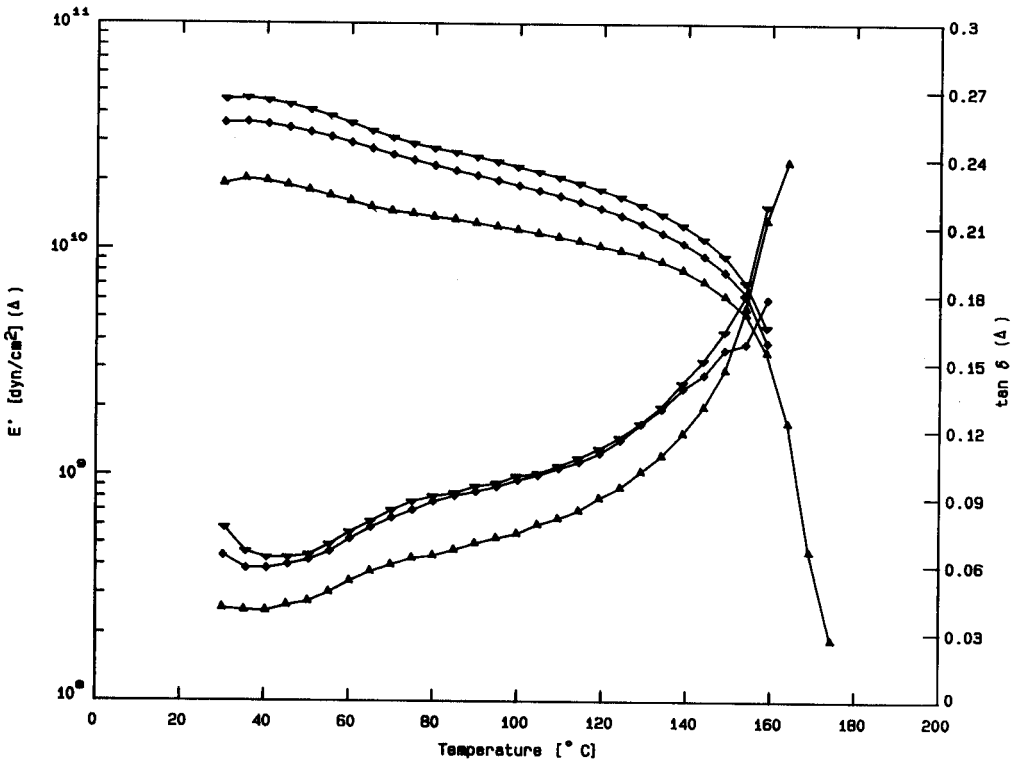

Figure 3.--*The variation in storage modulus (log E') and tan δ with temperature of polypropylene composites containing 50 wt. percent of (▲) unmodified, (◆) titanate modified, and (▼) fatty amine modified, red oak fiber.*

interaction for the latter two systems. Also common to all three spectra is the broad relaxation centered around 80 °C. The exact origin of this process is unclear at the moment, but may tentatively be assigned to the melting of small, imperfect crystalline domains. Of particular interest is the low temperature region of the tan δ curve where there is an additional relaxation peak that is unique to the fatty amine fiber composite and the unmodified fiber composite. This new transition is attributed to the wood fiber/polypropylene interfacial region and suggests improved compatibility between components.

From the observed T_m depression and the intensity of the low temperature transition, the degree of interaction can be ranked as follows: *TITANATE MODIFIED < UNMODIFIED < FATTY AMINE MODIFIED.* It is interesting to note that the impact strength of the composites, although exhibiting only small differences, followed a similar trend.

Phenolic Resin Cure. The curing analysis of thermosetting polymers presents a unique challenge due to the associated phase change from liquid resin to vitrified polymer. Although dynamic mechanical analysis has contributed extensively to this area of research (Aronhime and Gillham 1984), this method suffers in that some type of support (e.g., glass cloth) is required for the prepolymer. A relatively new version of the older dielectric analysis, microdielectric spectroscopy addresses this shortcoming. This technique incorporates new microsensor technology that can directly evaluate liquid samples and also provides the luxury of remote data acquisition. As a result, it affords a potentially powerful method for the study of thermosets such as phenol-formaldehyde polymers (Rials, in press).

The dielectric response as a function of temperature is compared with the reaction exotherm obtained by differential scanning calorimetry in figure 4. In this case, the dielectric data are plotted as ionic conductivity that is related to the mobility of ions in the medium and is, therefore, inversely proportional to viscosity. As the resin is heated, the conductivity increases rapidly until a slight slope change is observed at 65 °C, corresponding to the calorimetrically observed reaction onset. A conductivity peak, corresponding to the minimum viscosity, is reached at about 110 °C followed by a plateau region to about 140 °C. Interestingly, it is not until the DSC exotherm nears completion that a rapid decrease in conductivity occurs, indicative of increased crosslinking and network development. The crosslinking reaction exhibits a final advance as the experimental temperature exceeds that of the network polymer's T_g around 180 °C.

Microdielectric analysis of phenol-formaldehyde polymers yields information that is readily interpreted in very practical terms. Cure parameters such as viscosity minima, onset of reaction, rate of cure, and completion of reaction are easily extracted from the data. In addition, this technique has demonstrated considerable sensitivity to chemical structure variables relating hydroxymethyl phenol content to residual

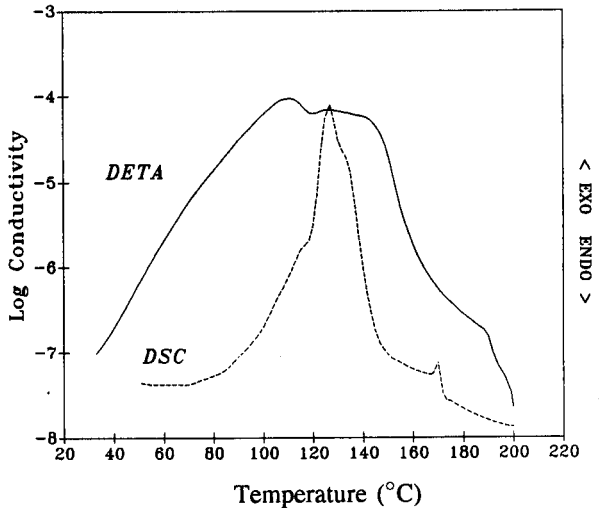

Figure 4.--*Comparison of the dielectric response (log conductivity) with the curing exotherm from differential scanning calorimetry of a phenol-formaldehyde resin.*

cure and illustrating the impact of unsubstituted *para* positions on the overall chemorheological response of the resin. In view of this capability, as well as the potential to collect data from remote environments, microdielectric spectroscopy can be expected to become as routine an analytical method as the other relaxation techniques.

CONCLUSIONS

Dynamic mechanical analysis and dielectric analysis have proved to be very useful in the study of polymer relaxation properties. In large part, their relatively rapid rise in popularity can be attributed to two unique features. First, these two analytical methods fill a critical void in terms of experimental time scale between transient experiments like creep and stress relaxation, and high frequency methods like nuclear magnetic resonance spectroscopy. The frequency range where they are applied allows the detection of large-scale molecular motion, as well as smaller, glassy state relaxation events. Perhaps the greatest strength of these techniques, however, lies in their unique capacity to relate molecular motion to polymer properties of immediate, practical significance.

The foundation of relaxation spectroscopy has been firmly established in the realm of polymer science, but its availability has helped to expand its scope of application to disciplines as diverse as food science and ceramics. The extension of dielectric analysis (and particularly, dynamic mechanical analysis) into wood science was inevitable as the consideration of wood as a polymeric composite has become increasingly common. As has been demonstrated above, understanding the viscoelastic behavior of wood may significantly impact the production of composite products like particleboard and fiberboard. A look to the future suggests an increased presence of synthetic polymers, beyond phenolic

resins, as novel thermoplastic composites are investigated. Given the demonstrated utility of both dynamic mechanical and dielectric analysis in studying the inherent properties of the components and the structural factors of the systems, their contributions to the science and technology of wood utilization will unquestionably increase.

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Use of Gel Permeation Chromatography in the Development of Resin Adhesives

Chung-Y. Hse

In a crude sense, gel permeation chromatography (GPC) is a molecular sieving process in which the polymer molecules are separated as a function of their size in solution. This separation technique provides the fastest and most accurate method for determining the molecular weight distribution of a polymer. Since the product uniformity, process characteristics, and physical properties of most polymers are related to the molecular weight (number average M_n and weight average M_w) and molecular weight distribution (M_w/M_n), the importance of this analytical determination can readily be seen.

The application of GPC in resin research at the Pineville laboratory began in 1967 with laboratory-assembled instruments (fig. 1). A typical chromatogram of a phenolic resin by this homemade instrument is shown in figure 2. The commercial GPC instrument was purchased in the early 1970's. The capability of GPC for polymer characterization has improved substantially in recent years with new instrumentation, techniques, and data manipulation. The resolution of phenolic species by GPC has improved significantly as shown in figure 3.

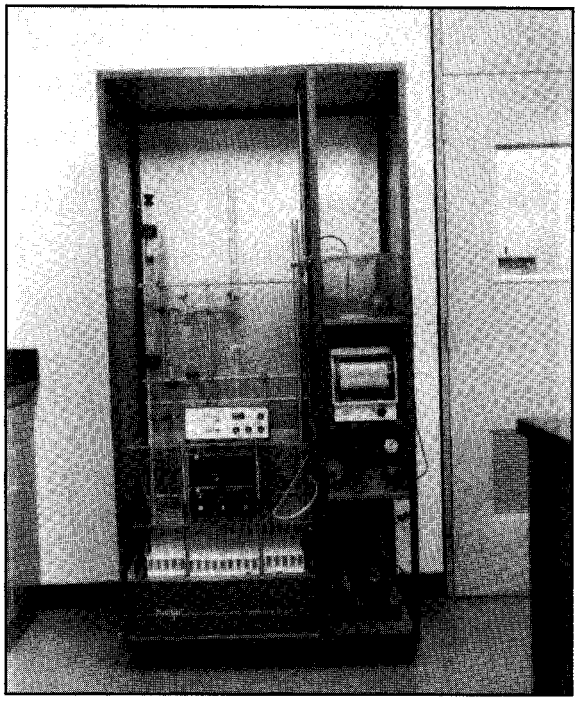

Figure 1.--*Front view of laboratory assembled GPC.*

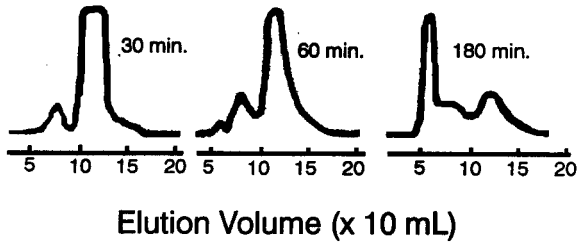

Figure 2.--*Gel permeation chromatograms of the NaOH catalyzed phenol formaldehyde resins at various reaction times.*

Over the years at the Pineville laboratory, GPC has been used in studies of resin formulation, characterization, molecular blending, resin storage and stability, fractionation and separation, and copolymerization. At present, GPC is used routinely to define molecular distribution profiles of resins formulated in the laboratory. The usefulness of gel permeation chromatography in the development of resin adhesives can be illustrated by the following examples.

To modify the urea formaldehyde (UF) resin formulation from the conventional two-stage reaction (i.e., alkaline pH to initiate the reaction and acidic pH to promote condensation) to a single acidic reaction without first using an alkaline catalyst, GPC was used to follow the reaction (fig. 4). The large fraction of sample eluted between 21 and 29 mL (upper chromatogram) indicated that strong acidic conditions favored condensation. On the other hand, the strong signal between 30 and 39 mL (lower chromatogram) showed that alkaline conditions enhanced methylol formation. The high methylol content in resins made under alkaline conditions as compared to acidic conditions was also detected by ^{13}C-NMR analysis (Hse and others, in preparation). In general, the chromatogram can be divided into two groups of peaks. The peaks between 39 and 40 mL elution volume are those of urea or mono-methylol urea, whereas the first negative peak between 41 and 43 mL is due to formaldehyde and water in the sample. These identifications were made by comparing GPC chromatograms of UF resins before and after addition of urea, formaldehyde, and water to the sample. Other peaks

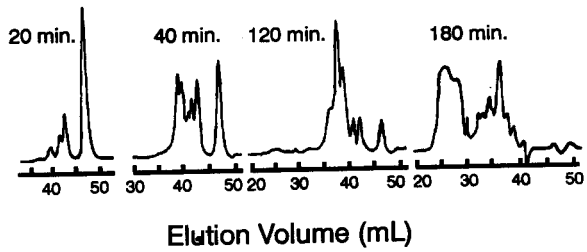

Figure 3.--*Gel permeation chromatograms of phenolic resins for flakeboard at various reaction times.*

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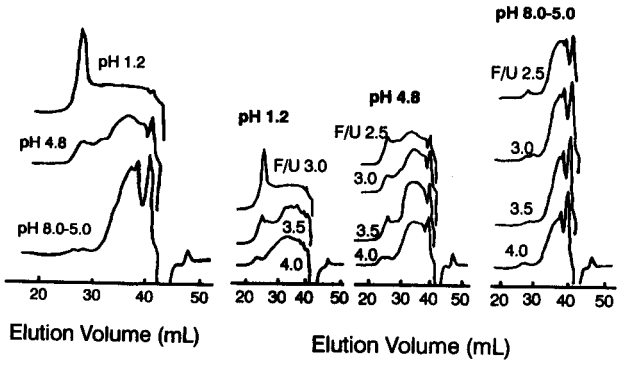

Figure 4.--*Gel permeation chromatograms of UF resins reacted at different pH and F/U ratios.*

evident at early stages of reaction (i.e., those between elution volumes 30 and 39 mL) may be dimers, trimers, and tetramers of polymethylol urea as indicated by previous workers (Dankelman and others 1982; Dunky and Lederer 1982; Matsuzaki and others 1980, Tsuge 1980). These peaks can be integrated together (group A) as an estimate of the amount of low molecular weight products. The peaks at low elution volumes (i.e., 21 to 29 mL) can be integrated together (group B) to represent a measure of the high molecular weight products obtained from condensation reactions.

The composition of low and high molecular weight products is summarized in table 1. The proportion of high molecular weight products increased substantially as the reaction pH decreased. Furthermore, the F/U molar ratios interacted with reaction pH to effect resin molecular weight. At acidic pH, the high molecular weight products increased as F/U ratio decreased; while, at alkaline pH, little difference was evident between the high or low molecular weight products at various F/U ratios. It should be noted, however, the molecular weight buildup was so fast for the resin made with an F/U ratio of 2.5 at strong acidic condition (i.e., pH 1) that an insoluble gel formed early, so control of the reaction was not practical.

The analysis of the molecular distribution profiles suggested that a very different resin was produced under strong acidic conditions because a high proportion of large molecular fraction was formed so quickly (i.e., 30 minutes) at such a mild temperature (i.e., 55 °C), yet the resins were extremely stable. NMR later confirmed the formation of a high percentage of uron derivatives that were not in the conventional UF resin. Furthermore, 2-hour desicator tests indicated that UF resin formulated with strong acidic pH also resulted in significantly lower formaldehyde emission even though the bonding strength was slightly lower than the conventional UF resin (Hse and others, in preparation). Preparative GPC is being used to fractionate the resins. It is expected that the IR and NMR studies of these fractions should provide more detailed structures of the resins.

The condensation polymers of phenol and formaldehyde as shown in figure 3 and UF as shown in figure 4 are the two most important wood adhesives. Phenolic resins have proven performance in producing exterior quality composites, whereas low cost UF resins have performed well in interior applications. Many users of wood adhesives have no doubt speculated on the advantages to be gained by blending phenolic and urea resins in searching for lower cost phenolic adhesives or for upgrading performance and controlling formaldehyde emission of UF adhesives. Efforts in

Table 1.--*Effect of reaction conditions on the proportion of low and high molecular weight species*

Resin	pH	Low molecular F/U ratio	High molecular weight products	Weight products
			----------percent----------	
A	1.0	3.0	54.7	45.3
B	1.0	3.5	78.2	21.8
C	1.0	4.0	88.8	11.2
D	4.8	2.5	70.5	29.5
E	4.8	3.0	79.8	20.2
F	4.8	3.5	88.2	11.8
G	4.8	4.0	92.3	7.7
H	8.0-5.0	2.5	94.8	5.2
I	8.0-5.0	3.0	96.2	3.8
J	8.0-5.0	3.5	97.0	3.0
K	8.0-5.0	4.0	96.1	3.9

developing these types of resin systems have not been successful due to the basic incompatibility of the curing properties of the two resins. Phenolic resins are normally cured at alkaline pH, whereas UF resins are cured at acidic pH. Therefore, in order to develop practical usage, it is necessary to combine urea to phenol-formaldehyde polymers as true co-polymers. Again, fractionation by preparative GPC was used to great advantage in examining the co-condensation of the model reaction.

Figure 5 shows the effect of reaction pH on the molecular weight distribution profile of the reaction products between 4-hydroxylbenzyl alcohol (PMP) and urea (Tomita and Hse, 1989) at the molar ratio of PMP/U = 1. It is obvious that the stronger the acidic condition, the shorter the reaction time to produce condensates. At pH 2.0, for instance, the oily reactant consisting mainly of hydroxybenzylurea began to separate from the water layer within 10 minutes. (The changes of gel permeation chromatography of the reactants reacted 30 minutes at pH 2.0 as affected by molar ratio of PMP/U are shown in figure 6.) Increased molar ratio (i.e., 2.0 and 4.0) accelerated the formation of the high molecular weight portion. Examination with NMR in a previous study (Tomita and Hse 1989) showed that the formation of the condensates involved co-condensation and self-condensation, with co-condensation between p-methylol group and urea prevailing against self-condensation of phenol.

On the other hand, at lower molar ratios (i.e., 1.0 and 0.5), GPC showed three separate peaks in the lower molecular weight region. The fractions of these three peaks were collected by preparative GPC. The chromatogram of each fraction is shown in figure 7. Again, the fractions were studied extensively with IR and NMR (Tomita and Hse 1989). The results revealed that peak 1 was crystallized 4-

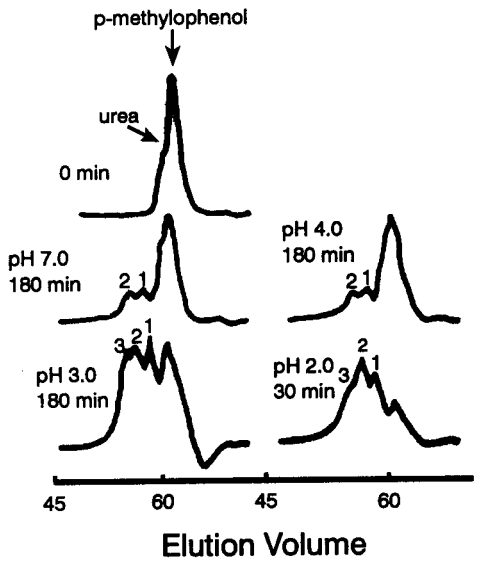

Figure 5.--*Gel permeation chromatograms of the reactant from urea and p-methylolphenol at the molar ratio of PMP/U = 1 and 85 °C, under various pH levels.*

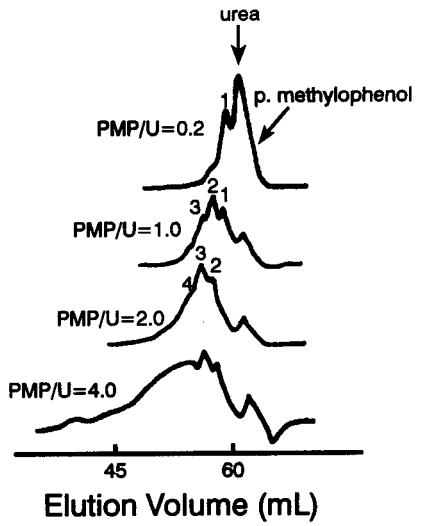

Figure 6.--*Gel permeation chromatograms of the reactants from p-methylolphenol and urea at various molar ratios (PMP/U). Reaction condition: 85 °C, pH 2.0, 30 min.*

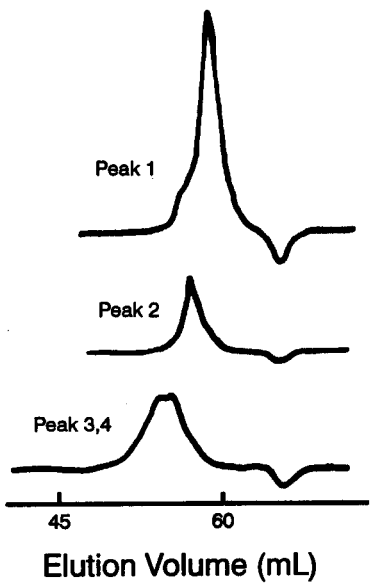

Figure 7.--*Gel permeation chromatograms of molecular fractions isolated by preparative GPC.*

hydroxybenzyl urea, peak 2 was crystallized N,N'-Bis(4-hydroxybenzyl) urea, and peak 3 was amorphous solid of Tris(4-hydroxybenzyl) urea.

The foregoing examples have clearly shown the utility of gel permeation chromatography as a separation technique for previously hard-to-analyze phenolic and UF materials. The effectiveness of GPC in wood adhesive research will grow

continuously, particularly in combination with other analytical methods such as NMR, DSC, and FTIR, etc. The ability of GPC to measure and evaluate the molecular weight distribution of polymer species should lead to better process control and more uniform products. It is expected that so-called "fingerprints" of wood adhesives may one day be realized for quality assurance, even though the wood adhesive is often noted for its complexity.

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Condensed Tannins: Must Enzymes Control the Stereochemistry in Profisetinidin Biosynthesis?

Jan P. Steynberg and Richard W. Hemingway

INTRODUCTION

The condensed tannins are polymeric flavan-3-ols that are found in the fruits, leaves, bark, and occasionally wood of a broad spectrum of plants (Hemingway and others 1989). Depending upon the plant species, these compounds vary in their hydroxylation patterns (for example, 3,5,7,3',4'-pentahydroxyflavans as in conifer tree barks or 3,7,3',4'-tetrahydroxyflavans as in wattle tree heartwood), stereochemistry (for example, predominantly 2,3-*cis*-3,4-*trans* in conifer tree barks or mixtures of 2,3-*trans*-3,4-*trans* and 2,3-*trans*-3,4-*cis* in wattle tree heartwood), as well as molecular weight distribution. The most common naturally occurring profisetinidins are polymers made up of 2**R**,3**S**-(-)-fisetinidol **(1)** as chain extenders with 2**R**,3**S**-(+)-catechin **(2)** as chain terminators.

Much effort has recently been devoted to a better understanding of enzymic pathways leading to condensed tannins, but debate continues (Stafford 1983, Platt and others 1984) regarding the exact nature of proanthocyanidin biosynthesis. Good progress has been made in understanding of the individual enzymic steps up to C-15 (monomer) level (Lewis and Yamamoto 1989, Stafford 1989), and more evidence for flavan-3,4-diols as the precursors to proanthocyanidins has been obtained (Stafford and others 1985). The ease with which these intermediates polymerize under very mild conditions has in some instances led to abandonment of hypotheses that invoke enzyme mediation in the polymerization process (Roux and others 1980, Roux and Ferreira 1982a, Roux and Ferreira 1982b). In contrast, our recent characterization of novel 2,3-*cis*-3,4-*cis*-profisetinidins (Steynberg and others, in preparation), as well as efforts to synthesize these compounds biomimetically, argue strongly in favor of the idea that the whole process of synthesis from the initial precursor to the ultimate polymer must be under enzymic control.

RESULTS AND DISCUSSION

The basic chemistry of the profisetinidin-rich wattle tannins extracted from *Acacia mearnsii* has been extensively studied by Professor Roux's group in South Africa, and this has led to successful industrial application of these tannins (Roux and others 1980, Roux and Ferreira 1982a, Roux and Ferreira 1982b). Much of the success obtained in these efforts can be related to the development of "biomimetic" synthesis, where these oligomers can be synthesized by mild acid-catalyzed condensation between naturally occurring flavan-3,4-diols and flavan-3-ols. This leads to the formation of an interflavanoid bond by the attack of the highly nucleophilic A-ring of catechin on the acid-mediated 4-carbocation of mollisacacidin (fig. 1).

As part of a search for North American sources of tannins suitable for leather manufacture, we investigated the bark tannins of a Mexican tree, *(Phytecellobium dulce)* (Steynberg and others, in preparation). Two novel 2,3-*cis*-3,4-*cis* profisetinidin dimers **(3)** and **(4)** dominated the dimeric fraction (fig. 2). The structural elucidation of these unique compounds relied mainly on physical data obtained by powerful n.m.r. techniques such as selective decoupling and n.O.e. difference spectroscopy that again stresses the need to have modern n.m.r. instrumentation available in a study of relatively complex molecules like tannins. Diagnostic ^{13}C resonances indicated 2,3-*cis* stereochemistry for both extender units as well as for one of the terminal units. The magnitude of the heterocyclic ring proton coupling constants for the methyl ether acetate derivatives, assisted by ^{1}H-decoupling experiments and n.O.e. difference spectra, not only confirmed the relative stereochemistry at C-2 and C-3 (2,3-*cis* in the chain extender units and either 2,3-*trans* or 2,3-*cis*, respectively, in the bottom units), but also indicated 2,3-*cis*-3,4-*cis* relative stereochemistry for the upper units. Absolute stereochemistry at the interflavanoid bond was derived from a positive Cotton effect in the CD-spectra of both dimers. The absolute stereochemistry of the bottom unit in **(3)** was evident from optical rotational measurements of the terminal flavan-3-ol obtained after interflavanoid bond cleavage. Collectively, these data confirmed the suggested structures **(3)** and **(4)**.

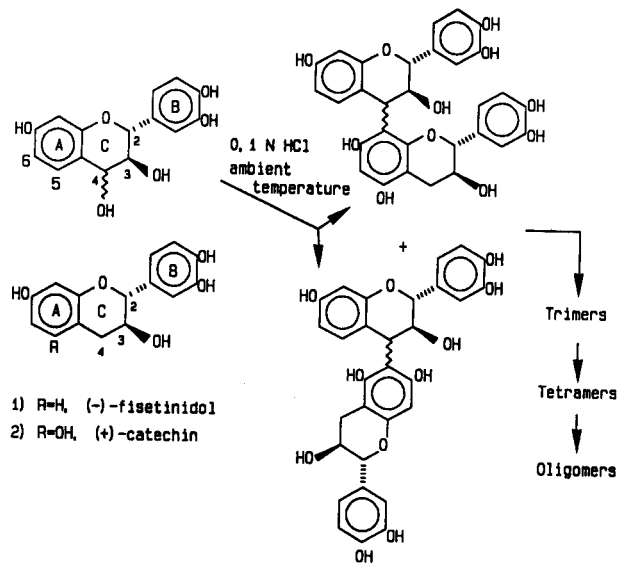

Figure 1.--*Biomimetic synthesis of profisetinidins.*

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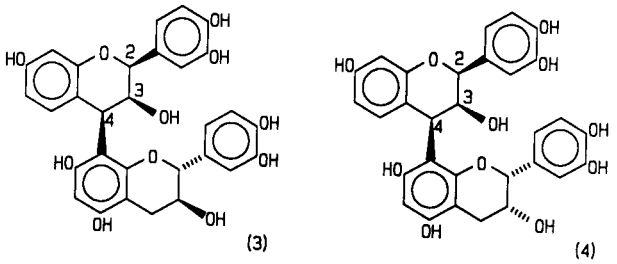

Figure 2.--*Novel all-cis profisetinidin dimers from Guamuchil.*

Not only do these dimers contribute to the rare class of 2,3-*cis* profisetinidins, but in all known proanthocyanidins, there has been only one other natural product isolated with a 2,3-*cis*-3,4-*cis* stereochemistry (Foo 1987). Attempts to obtain these compounds via acid-catalyzed condensation (Roux's methodology) was severely limited in two ways. A 2,3-*cis* mollisacacidin is extremely rare, having been isolated in only very small quantities from *Acacia mearnsii* (Drewes and Ilsley 1969) and is not easily obtainable by synthesis. Additionally, 2,3-*cis*-flavan-3,4-diols will undergo stereospecific reaction to yield only 3,4-*trans* products when exposed to mild acid-catalyzed condensation reaction conditions (Botha and others 1981). Therefore, an obvious hypothesis for how these all-*cis* compounds might be formed was to suggest that they originate from a *trans-cis* profisetinidin precursor by epimerization at C-2. All tannin chemists agree that any suggested biochemical conversions should be demonstrated to be possible on solid chemical grounds, so we attempted this synthesis.

Epimerizations of (+)-catechin and (-)-epicatechin have been successfully accomplished by treatment with either strong base or high temperature (fig. 3a) (Foo and Porter 1983). In these reactions, opening of the pyran ring to a quinone-methide intermediate **(4)** destroys the absolute stereochemistry at C-2. Ring closure via *Re*- as well as *Si*-face attack of the A-ring oxygen nucleophile on the quinone-methide results in reaction products with both 2**R** and 2**S** absolute stereochemistry. However, the ease with which profisetinidins undergo pyran ring isomerization to phlobatannins restricts approaches to use of epimerization reactions in oligomeric compounds. Here the terminal unit's A-ring hydroxyl acts as a competing nucleophile in the ring closure step to produce a different pyran ring with "inversion" of the A- and B-rings (fig. 3b) (Steynberg and others 1988). Therefore, tetra-*O*-methyl catechin **(5)** was reacted with mollisacacidin to synthesize selective protected dimers **(6)** and **(7)** suitable for these attempts (fig. 4). By protecting the bottom unit's hydroxyls as methyl ether derivatives, not only are pyran ring isomerizations prevented, but also the bottom unit is protected from unwanted epimerization.

Although it was possible to epimerize the *trans-trans* derivative **(6)** at C-2 by heating it in ethanol under pressure

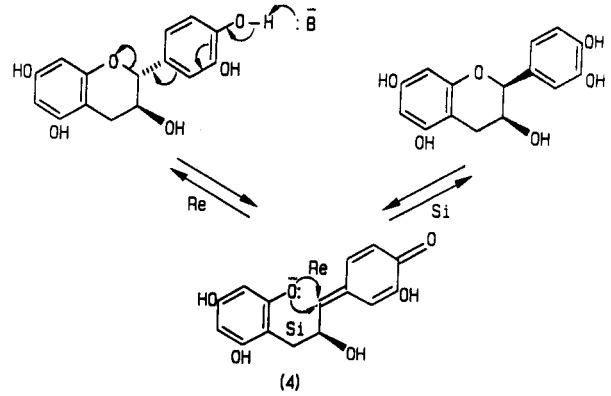

Figure 3a.--*Quinone-methide intermediates in profisetinidin synthesis.*

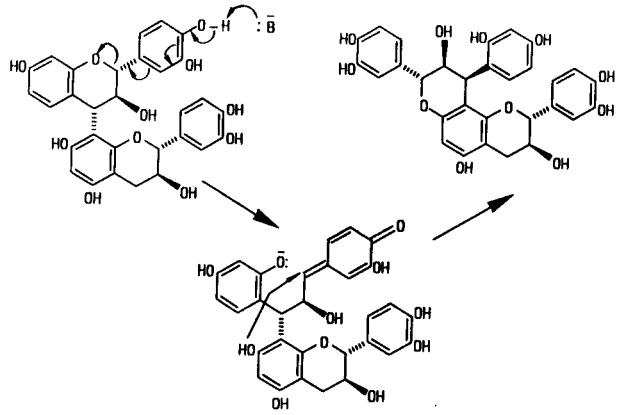

Figure 3b.--*Quinone-methide intermediates in profisetinidin synthesis.*

at 180 °C, all efforts to epimerize the *trans-cis* dimer **(7)** resulted only in recovery of the starting compound. This might be explained in terms of a stereospecific ring closure directed by the bulky 4-substituent occupying an axial orientation as is the case with the *trans-cis* dimer **(7)**. In the quinone-methide intermediate originating from this compound, orientation of the quinone above the mean plain of the A-ring, a conformation required for attack that will lead to epimerization, may be very unfavorable due to severe steric interaction between the quinone and the bulky *quasi*-axial 4-substituent. The α-orientation of the 4-substituent (below the mean plane of the A-ring) in the *trans-trans* dimer **(6)** makes such stereocontrol impossible in this case and explains why **(6)** does epimerize under high temperature conditions.

CONCLUSIONS

It is most unlikely that the all-*cis* profisetinidins isolated from Guamuchil are produced in the plant from 2,3-*trans*-3,4-*cis* precursors such as fisetinidol-(4ß → 8)-catechin

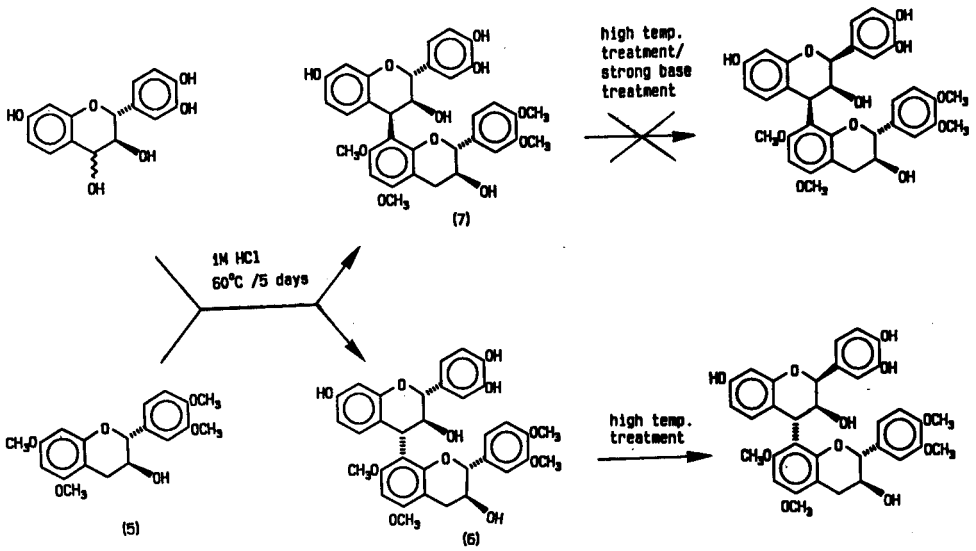

Figure 4.--*Attempts to synthesize the all-cis profisetinidin dimers via epimerization of a suitable precursor.*

or fisetinidol-(4ß → 8)-epicatechin by an epimerization at C-2 because fisetinidol-(4ß → 8)-tetra-*O*-methyl catechin cannot be epimerized to the 2,3-*cis*-3,4-*cis* ent-epifisetinidol-(4ß → 8)-tetra-*O*-methyl catechin. These results together with the facile formation of phlobatannins suggest that the 2,3-*cis* stereochemistry must be established prior to the formation of the flavan-3,4-diol precursor. Therefore, enzymatic control, both in the formation of a 2S,3S precursor and stereoselective formation of the 4ß interflavanoid bond seems to be required in the biogenesis of these compounds.

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Can Wood Extractives be Used as Wood Protectants?

C. A. McDaniel and Billie S. Dunn

INTRODUCTION

It is estimated that the cost of the control and prevention of subterranean termites and the repair of damage they cause is approximately 1.5 billion dollars per year in the United States (Ebeling 1975). This estimate is from data in the 1960's and, because of inflation, the present cost is probably much higher.

Investigators have shown that certain woods demonstrate a natural resistance to termite attack (Beal and others 1974; Carter 1972, 1974; Carter and de Camargo 1983; Carter and Huffman 1982; Carter and Smythe 1972, 1974; Carter and Stringer, 1976; Carter and others, 1972, 1976; Smythe and Carter, 1970a, 1970b). Our investigations involve the isolation and identification of the antitermitic compounds extracted from the heartwood of these resistant woods, as they present possible environmentally safe alternatives to currently used synthetic wood preservatives (McDaniel 1989; McDaniel and others, in press).

METHODS AND MATERIALS

Heartwoods from species that have previously shown resistance to termite attack in laboratory tests are ground into sawdust and exhaustively extracted with a ternary solvent mixture consisting of 54:44:2 acetone:hexane:water (v:v:v). The sawdust is bioassayed before and after solvent extraction in order to insure that the antitermitic activity has been removed from the wood into the solvent extract. The total extract is chemically fractionated into acidic, phenolic, and neutral fractions by successively washing an ether solution of the extract with 4N sodium bicarbonate, followed by a 1N solution of sodium bicarbonate, followed by a 1N solution of sodium hydroxide.

Since antitermitic extracts typically contain a mixture of compounds, some of which have antitermitic activity while others do not, separation must be achieved into pure compounds that may then be bioassayed individually. These separations are achieved by a variety of chromatographic methods including thin-layer chromatography, column chromatography, and primarily by high performance liquid chromatography (HPLC). Most of our separations are achieved on a Perkin-Elmer 3B Liquid Chromatograph using a reverse-phase HPLC on a Whatmann M-9 ODS-E semipreparative column. A typical solvent system employed is a 60:40 (v:v) acetonitrile:water mixture with a Hewlett-Packard 1037A refractive index detector monitored with a Hewlett-Packard 3392A integrator. The fractions are collected, partitioned into methylene chloride, and the solvent removed in vacuo at 40 °C. Purities and component identities are primarily determined by capillarilly gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 5890-A GC interfaced to a 5970 Mass Selective Detector, a 9000-216 computer, and a 9133 disk drive.

Bioassays. After sufficient quantities of materials have been collected in acceptable purities (> 95 percent) the isolates are bioassayed for efficacy as wood protectants. The materials are vacuum-impregnated into either sweetgum *Liquidambar styraciflua* L. or southern pine (Pinus spp.) blocks according to ASTM methodology (1976) at concentrations of 1 percent and 0.5 percent by mass. In this way, the compounds responsible for antitermitic activity can be assessed.

Two protocols are employed in laboratory bioassays: the no-choice test and the choice test.

In the no-choice test, a mixture of sand:vermiculite:water (1:1:0.4 by volume) is placed into a cylindrical plastic test container that measures 4.0 cm high by 5.3 cm in diameter. A treated wooden block is carefully weighed and buried in the test chamber, and 100 worker termites of the eastern subterranean termite species *Reticulitermes flavipes* are then added. Blocks treated with solvent alone serve as controls. After 4 weeks, the block is removed, ovendried at 60 °C for 4 hours, and reweighed. It is also visually inspected, and a block rating is assigned (table 1) based on weight loss and the extent of termite damage. Surviving termites are counted, and their physical condition is noted.

The choice test is similar except that two test chambers connected by a plastic foraging tube 0.5 X 10 cm are used. The treated wooden block is placed into one chamber along with the termites, and an untreated wooden block is placed in the second chamber. The termites then have an alternative habitat and food supply available to them, and they may avoid the treated block without facing starvation, and therefore, any repellent or antifeedant activity may be evaluated. As with no-choice tests, the blocks and termites are examined at the end of a 4-week period.

Table 1.--*Grading scale for vacuum-impregnated wood*

Number assigned	Physical appearance	Dry weight loss
		percent
0	no apparent attack	0 - 2.0
1	surface nibbling	2.1 - 5.0
2	moderate attack	5.1 - 20.0
3	heavy attack	20.0 - 50.0
4	very heavy attack	>50.1

C.A. McDaniel is principal scientist and Billie S. Dunn is physical science technician at Southern Forest Experiment Station, USDA Forest Service, Gulfport, MS 39505.

Those compounds that exhibit antitermitic activity in laboratory bioassays are vacuum-impregnated into either stakes of sweetgum measuring 15.5 X 0.75 X 3.2 cm or into sweetgum blocks measuring 2.3 X 3.9 X 0.75 cm, which are then stapled onto untreated stakes having the above dimensions. The blocks are employed in situations involving minor components where difficulties arise in isolating sufficient materials to treat entire stakes. The stakes and/or blocks are impregnated at levels of 1.0 and 0.5 percent by mass and field-tested for 1 year. The rating system previously described for laboratory tests (table 1) is used to evaluate the test materials at 6 months and at 1 year.

The following isolates have been field-tested: α-terpineol (Port-Orford-cedar), cedrol (eastern redcedar), and catalpalactone (southern catalpa). Sesquiterpenoids available from other sources that have been tested are: citral, citronellal, geraniol, nerolidol, and farnesol. Current materials in field tests are: eastern redcedar leaf oil, occidentalol, occidol, and eudesmol isomers, all from northern white cedar, and finally, an acelylated derivative of a wood waste product from Hercules Chemical Corporation called Vinsol.

RESULTS AND DISCUSSION

For all wood species examined, the preliminary tests on cellulose pads (some of which are shown in table 2) indicated that the acidic and phenolic fractions possess little or no antitermitic activity. The hydrocarbon sesquiterpenes and terpenes likewise were found to be innocuous, or nearly so, to termites at the levels tested. The activity was found to be in oxygenated terpenes and sesquiterpenes. Results of some of these tests are shown in table 3.

Table 2.--*Toxicity to* Reticulitermes flavipes *of cellulose pads treated at 0.5 percent with some heartwood-extracted materials*

Sample extract	Percent Survival
Port-Orford cedar	
neutral extract	0 (2 days)*
sesquiterpene hydrocarbons	0 (1 day)
α-terpineol	0 (1 day)
85 percent α-cadinol/ 15 percent torreyol	0 (2 days)
T-muurolol	0 (4 days)
Southern catalpa	
neutral extract	0 (10 days)
phenolic extract	95
catalponol	0 (7 days)
epicatalponol	0 (21 days)
catalponone	0 (3 days)
catalpalactone	0 (28 days)
Western redcedar	
neutral extract	0 (2 days)
acidic extract	92
phenolic extract	100

*Days to mortality

Table 3.--*Antitermitic activity towards* Reticulitermes flavipes *of some heartwood extracted materials in susceptible woods at 0.5 percent*

Sample extract	Percent survival choice (block rating)	No choice (block rating)
Western redcedar		
neutral extract	85 (0)	50 (1)
phenoli extract	73 (1)	87 (2)
acidic extract	92 (2)	95 (2)
methyl thujate	82 (1)	84 (2)
Southern catalpa		
catalponol	*	82 (2)
epicatalponol	*	72 (2)
catalponone†	*	68 (2)
catalpalactone	*	0 (0)
Eastern redcedar		
total extract	†26 (1)	85 (2)
cedrol	*	50 (1)
Port-Orford-cedar		
α-terpineol	*	0 (0)

* Test not performed.

† Pathogenic fungus present in test container.

But this cellulose pad test bioassay was found not to be a reliable indicator for our purposes. It indicated toxicity, but did not show which compounds would protect woods from attack, the main thrust of our investigation. Vacuum-impregnating the test materials into susceptible woods and bioassay of this treated wood were chosen as the indicators for this purpose. Some results are shown in table 4. The results of these bioassays sometimes differed from the earlier pad bioassay. Catalponol, for example, showed greater toxicity in pad tests than did catalpalactone; but catapalactone protected wood from attack better than did catalponol, its epimer epicatalponol, or the sesquiterpene acetone, catalponone.

Our field bioassays (table 4) indicate that the laboratory bioassays are reliable, but also that, in the extended weathering and higher termite pressure of field conditions, higher levels of treatment are probably necessary. Future studies will reflect treatment levels at or above those found naturally in resistant heartwoods.

Table 4.--*1-year protection of susceptible woods in a woodland environment by treatment with heartwood-extracted materials at 1.0 percent*

Sample extract	Rating ± SC (10 replicates)
Port Orford-cedar	
total extract	0.56 ± 0.88
α-terpineol	0.56 ± 1.33
Eastern redcedar	0.78 ± 1.20
cedrol	0.11 ± 0.33
Southern catalpa	
total extract	0.25 ± 0.70
catalpalactone	0.11 ± 0.33
Control	0.63 ± 1.06

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*U.S. GOVERNMENT PRINTING OFFICE:1994-565-016/80052

Rials, Timothy G., ed. 1994. Symposium on current research in the chemical sciences: Proceedings of the 3rd annual Southern Station chemical sciences meeting; 1990 February 7–8; Alexandria, LA. Gen. Tech. Rep. SO-101. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 63 p.

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