CHANGES IN PLANTS DURING LOW TEMPERATURES.

Part I. Oil Synthesis at various elevations of the Himalayas and its Physiological Influence on the Protoplasm of Cedrus Deodara

BY

R. C. Malhotra

Professor of Biology, St. Mary's College, St. Mary's, Kansas, U.S.A.

Introduction.

Within recent years, winter injury to plants in general, and fruit trees in particular, has received a great deal of attention by botanists. As a result of such studies, some data have been accumulated pertaining to the cause of direct death or injury of plant protoplasm due to freezing. In spite of these noteworthy researches, so many conflicting views have arisen from some of these conclusions, that any factor, single or complex, needs further experimentation before its acceptance or contradiction.

In many results reported due to freezing injury, only artificial conditions have been induced. Any chemical, cytological, histological or physical changes produced during these environmental conditions have been attributed, in the main, as an evidence of death or injury by direct freezing. However, such a generalization may or may not be true. Data will be presented in one paper of this series, which will show justification of these remarks.

It was therefore proposed, that critical study be made to find the mechanism of death or injury, in plants, directly due to freezing. In order to do that, first normal changes in nature and their significance in laboratory were followed. It is hoped, that any information derived from chemical, physiological and anatomical study, may either confirm, add or change the views held previously.

The present paper is an outcome of the first of the series. It may be of interest not only to botanists, but also to horticulturists and agronomists, because, in popular literature with reference to freezing injury in orchards, vegetable gardens and crop fields, advice to growers concerned is often based upon erroneous notions or inconclusive evidence as to how freezing kills.

Review of the Literature.

Sachs (54), Muller-Thurgau (42), Molisch (40), Voigtlander (59), Wiegand (61), and many others have shown, that plant cells are not injured without ice formation. It may be formed within the cell or in the intercellular spaces, usually in the latter. When the tissue is at the freezing point or below, ice is not formed throughout the surface at the same time. According to Muller-Thurgau, there are points, where crystallization begins, and the water from adjoining and distant intercellular spaces moves to these points. In other words, water is released for ice formation, because it cannot be held by imbibitional pull of protoplasm, cell walls, vacuoles and protoplasmic surfaces. That water is held by imbibitional pull has been shown by Shull (55).

Rosa (51), (52), (53), Hooker (15), (16), and Newton (46) have shown the importance of pentosans against winter injury. They seem to think, that these chemicals being of collidal nature, hold water against ice formation. Many workers, particularly those in America, such as Chandler (3), Gardner, Bradford and Hooker (9), agree with the pentosan theory of cold resistance. Chandler in summarizing this problem, writes thus, "The presence in the protoplasm of colloidal substances, such as pentosans, seem to be of more importance; probably because such substances increase the colloidal stability of the protoplasm and thus increase its ability to withstand ice formation in the tissue."

Doyle and Clinch (5), based on data of extensive researches, do not seem to find any relationship between the pentosan content and the relative hardiness of different types of conifers, nor between pentosan content and seasonal hardiness. They remark thus, "The theory is considered unnecessary and unimportant." Lidforss (27), (28) claims, however, that winter green leaves (as conifers possess) are starch free during the cold period. If this is true, the mechanism of cold injury in conifers and in broad leaf trees may be entirely different. Results of Lidforss have been questioned by Doyle and Clinch, who present experimental support of Lewis and Tuttle (25), Tuttle (58) and Miyake (39). They claim, that even in conifers, there is an increase of sugars during the cold months, associated with a diminution of starch content.

If this be granted, that pentosan theory of cold resistance is applicable to plants other than conifers only, even then there seems to be little support for it. Malhotra (33) recently presented data, which seem to show, that in the four vegetable crops used in his experiment, the total pentosans rather than their percentages, as advocated by Rosa and Hooker, may be important, if at all. He further brought out, that the size of cells and their compactness may be of greater significance than chemical differences in this connection. Furthermore Sinnott (56) points out, that winter food reserve changes cannot be brought about in summer by lowering of temperature, but develop in autumn while the environment is far from harsh, not only in trees of northern temperatures, but also in the trees of the frostless areas of the Gulf States of America.

Several authors such as Murneek (44), Clements (4) and Roberts (50) seem to think, that hemicellulose may be of some importance in its relation to winter resistance of plants. Two of these authors, however, have not secured any direct data to this effect. On the other hand, Gardner (8) could not find any effect of the different temperature conditions on non-hexose reducing substances, water soluble polysaccharides other than starch, fat and total nitrogen. Data obtained by Malhotra (33) show results similar to Gardner's with reference to changes in hemicelluloses in vegetable plants grown under low temperatures.

Gaumann (11) followed seasonal changes in carbohydrate content of spruce and fir trunks. He found, that carbohydrates (sugars and readily hydrolyzed polysaccharides) of the sap wood of Picea excelsa were 17.2 and 15.9 per cent in April and October (maximum) respectively; while they were 6.9 and 11.8 per cent in February and July. In heart-wood, they were -11 per cent in September and 8.47 per cent in March. The carbohydrates of sap-wood of Abies pectinata were 15.5 per cent in November and 15.3 per cent in April, while they were 7.7 per cent in February and 11.3 per cent in June. As to heart-wood, they were 10 per cent in August and 5 per cent in November. The total aqueous extract of heart-wood in both trees was nearly constant; while in sap-wood, it was higher in March and June than the rest of the year. These results do not seem to show any definite trend of carbohydrates and temperature changes in nature.

From the literature presented above, the rôle of pentosans and hemicelluloses as advocated by some authors, does not seem to be conclusive, even doubtful. On the other hand, several authors seem to think that starch is transformed into oil, at least in conifers, during winter. This oil forms an emulsion, which precools at a comparatively lower temperature before ice formation. This way the plant can escape winter injury. Thus, according to them, the greater the quantity of oil, the greater the protection against freezing.

It has been shown by Muntz (43), Leclerc du Sublon (23), (24), Gerber (12), (13), Ivanow (19) and others, that in many seeds, accumulation of oil is accompanied by a corresponding decrease in carbohydrates. Under proper conditions, this transformation takes place in ripe seeds detached from the mother plant, further indicating that the oil is derived from the carbohydrates.

Keith (22) obtained some data, which suggest, that toxic action of ice is reduced by fats and oils. When bacteria, in his studies, were held at $-20°C$ in water containing 5 to 45 per cent glycerine, a large percentage remained alive for many months; while at $-20°C$ in tap water, nearly all were dead in five days and all within a few weeks Furthermore, most vegetable fats and oils have lower freezing and higher boiling points, than either water or dissolved substances such as plant sap. This has been accepted by many chemists as recorded by Mathews (38).

Oils and fats are not evenly distributed in the plant world, not only as to species but also within a species. Garner, Allard and Foubert (10) write thus: "As a consequence of the physiological relationship of oil to carbohydrate, it appears, that maximum oil production in the plant required conditions of nutrition favorable to the accumulation of carbohydrate during the vegetative period and to the transformation of carbohydrate into oil during the reproductive period." These authors further point out, that climate is a more potent factor than soil type in controlling the oil content, at least of the seed. On the other hand, Nageli and Cramer (45), as a result of extensive researches, do not seem to find any relationship between oil distribution and climate.

Recently McNair (29) has reviewed oil and starch content of seeds as to their taxonomic and climatic distribution. He concludes, based on no experimental work, that because a large percentage of oil occurs in the seeds of the tropical and temperate plants, it may function as a protection against rapid temperature changes. He further states, that increased frequency of oil in the seeds of temperate plants is in accordance with the increased fuel value of oil over starch. As a proof he attempts to present chemical and physical properties of oils. For instance, he states that, "As oil conducts heat less rapidly than starch, it may, therefore, be of service in the seed as a protection against cold temperatures in temperate climates or against excessive heat in the tropics."

When McNair's review is examined critically, it presents many difficulties. If quantity of oil rather than kind of oil (the latter is not mentioned by this author) acts as a protector against cold, then the tropical plants should have the least amount of oil; while the temperate plants should have maximum oil content. Yet this is not true in nature. Furthermore, oil is utilized but very little during germination as some data obtained by Malhotra (35) seem to indicate. In vitro, carbohydrates, especially pentosans can stand more low temperature without disorganizing the cells than oils. It seems to the writer, that if oils or fats have any influence against winter injury to plants in temperate regions, it may be entirely due to the kind of oils as also stated by this author (30). The quantitative synthesis of oils in species or within the species growing under different environments, may be quite independent of the climatic effect. Some data will be presented in this paper, which may substantiate such a view point.

In summarizing the role of oils or fats in plants against winter injury, it may be said, that our knowledge is far from being complete in this respect at the present time. However, there is a need of further elucidation of this problem.

Materials and Methods.

Cedrus deodara, commonly known as deodar was selected, because it is the most durable conifer of the Himalayan woods as remarked by Gamble (7). It does not possess resin passages (very few if any) as noted by Howard (17). Resins may not have an effect similar to oils and fats, at least in its relation to protection against freezing. Furthermore, deodar possesses a wide range of growth. According to Troup (57), it grows between 4,000 to 10,000 feet above the sea-level. This margin in the elevation is great enough to change temperature considerably. It is true of sudden fluctuations as well as of the probable range as verified by going over the meteorological record of Udhampur district, from which these trees were selected. The heart and sap-woods of deodar are very prominent. The former is yellowish brown and the latter is white. There is a definite demarcation at the line of transformation.

Four trees were selected at the elevations of 5,000, 7,000, 9,000 and 10,000 feet from Batot to the end of Kishtwar forests, Udhampur district, Jammu Province, Kashmir State, India. The choice of this territory was made due to two reasons. First because, it lies in the Himalaya mountains, probably the steepest of all the mountainous regions of the world. Thus a maximum environmental change could be secured with a minimum distance. Second because, this range is practically of the same geological origin. The mechanical and chemical composition of the soil varies but very little. Morrison (41) also seems to bring forth this fact. The accompanying map shows the probable location and elevation, where these trees were selected.

Samples of the woods were taken during early part of January. There are at least 3 points, which must be considered in obtaining a representative wood sample for analysis, namely, portion of the tree from which the wood is taken, uniformity of the sample and optimum size of wood particles of the sample (14)

Map of Kashmir State, India, showing the Approximate Location and Elevation, from which Deodar Woods used in these experiments were obtained.

A portion of tree, 20 feet above the ground was chosen because the wood is uniform there. Jhonsen (20) has shown difference in analysis due to this factor. Sap and heart-wood samples were collected separately. It was shown by Ritter and Fleck (49) that there is a difference in the cellulose, lignin and oil content of heart-wood and sap-wood in the same species. Sap-wood is least resistant to winter injury. Chandler (3) says, that when a tree has gone into winter with all tissues well matured, the sap-wood is often less resistant than heart-wood.

Each sample of wood obtained was about 3 inches thick in the form of a cross-sectional disc, which was split into 2 portions. From each one of these portions, shavings not exceeding 0.005 inches thick were secured. Before shaving, the sample was dried at 100°C to kill all the enzymes present, since the same samples were proposed to be used for carbohydrate and protein analysis. The shavings were dried at 80°C for 48 hours, ground in a grinding machine manufactured by Enterprise Mfg. Co., Philadelphia, Pa. and divided into 2 portions (1: 3). The smaller portion was pulverized to 60 meshes per inch according to Malhotra (34), which was used for quantitative analysis of all fractions. Jhonsen and Harvey (21) have advocated the use of 100 meshes, but at this degree the sample gives less yield. Furthermore, at such fineness, cellulose breaks down as shown by Mahood (31) and Mahood and Cable (32). The samples were dried at 80°C to constant weight. Since dry-wood is highly hygroscopic, it was immediately transferred into glass stoppered bottles and put in desiccator to cool. In every case, results were calculated on the oven dry basis.

The larger portion was pulverized to 30 meshes per inch. From this whole portion, oil was extracted, which was later used in studying the chemical and physical properties of oils secured.

About 3 grams samples, in duplicate, were weighed for the estimation of fats and oils. This weight was found desirable by Malhotra (34). It was extracted with petroleum ether by means of Soxhlet apparatus following the direction of Lewkowitsch (26). Petroleum ether was used for strictly accurate result, because it does not dissolve the oxidized acids and thiobromine (2).

Saponification value was determined according to Lewkowetsch. A blank for reagents was run. The results were expressed as milligrams of potassium hydroxide requisite to neutralize the total fatty acids in one gram of the sample. Allen (1) has proposed to calculate the number of grams of fat saponified by one equivalent of KOH, that is, by 56.1 grams of KOH. However, many chemists such as Lewkowitsch (26) think that there is no advantage in this form of expression.

Viscosity was measured by Engler's (6) viscosimeter. Redwood (47) introduced a correction by multiplying the result by the specific gravity of the sample and dividing by 915. Since, however, there is no correlation between specific gravity and viscosity, there does not seem to be any need for a correction. 50 cc. of oil and fat was used in each case at constant temperature of 20°C.

Iodine value, which is a measure of the proportion of unsaturated fatty acids was determined by Hubl process (18). Wijs' modification of Hubl method was not tried because it was considered not to yield any better results in this particular case.

Reichert value was estimated by Richert's (48) process as modified by Wollny (63). A blank was run in order to determine the value of the reagents. Most of the analyses were calculated after Wilkinson (62).

Presentation of the Data and Discussion.

Results have been reported in Table No. 1. Figures in column 2 seem to indicate, that the percentage of oils (ether extract) in sap-wood has increased at 7,000 ft., but has decreased at 9,000 ft. and has increased again. The oils of heart-woods seem to be the same at 5,000 and at 7,000 feet, but have increased quite materially at 9,000 ft. and finally have decreased again. The data further seems to bring out, that on the whole, sap-wood contains less oil than heart-wood.

Many writers have pointed out, that sap-wood is less resistant to cold than heart-wood. Malhotra (37) found, that sap-wood of many varieties of pomes, nuts and conifers was killed when heart-wood was not injured at all. If there is any relationship between the percentage of oils present and the cold resistance, no such evidence seems to have been brought forth by these figures. On the other hand, the data seem to indicate, that oil may vary not only in the heart and sap-wood, but also at various elevations. There does not seem to be any definite tendency shown by deodar towards its oil content with the increasing elevation. It may also be pointed out, that the meteorological record of the last 15 years shows, that there is a decided direct relationship between the higher elevation and low temperature at these points.

It is unfortunate, that much data is not available, which may indicate the average percentage of oils in most conifers. This field remains yet unexplored, because commercial oils are found only in seeds and fruits.

Weichmann (60) extracted fixed oils from Bass-wood grown in Michigan. Its yield was somewhat lower than that of Deodara.

There is one more point, which the data presented in column 2, seem to bring out. The difference in the percentage of oils either between sap and heart-woods (except at an elevation of 9,000 ft.) or at various elevations is not a pronounced one. This is particularly true, when experimental errors are taken into consideration.

Table I.—Showing the Percentage of Oils and Fats found in Cedrus Deodara Woods at Various Elevations and their Physical and Chemical Properties.

Elevation in feet and the kind of wood.	Percentage of oil.	Iodine value.	Saponification value.	Reichert value.	Viscosity seconds for outflow of 50 cc. at 50°C.
I	II	III	IV	V	VI
5,000					
Sap-wood ...	5.9	101.3	189.1	2.6	415
Heart-wood ...	6.1	101.9	192.6	2.9	427
7,000					
Sap-wood ...	6.1	101.4	195.4	3.1	429
Heart-wood ...	6.1	102.2	195.8	2.6	432
9,000					
Sap-wood ...	5.3	101.6	201.6	4.6	430
Heart-wood ...	7.9	102.9	105.1	3.9	435
10,000					
Sap-wood ...	6.3	109.7	207.1	2.7	473
Heart-wood ...	6.4	113.0	207.2	2.9	473

Graph No. I illustrates the effect of elevation on the percentages of oils and fats found in the sap and heart-woods.

Column III shows the iodine value of the oils extracted from these woods. The iodine value increases but slightly with elevation for both kinds of wood, except at 10,000 feet, where it has gone up considerably. The woods obtained from this elevation were grown under the coldest temperature found in the trees of this study. It therefore appears, that there may be some relationship between the degree of cold and the iodine value, at least in Deodar. Since iodine value is a measure of the proportion of the unsaturated fatty acids, it seems that the Deodar wood synthesised more unsaturated oils with higher elevation or low temperature.

Saponification values presented in column IV indicate, that they increase or decrease irregularly as to the elevation and the kind of wood, although there is a sudden increase at 10,000 feet. Sap-wood shows lower saponification at 5,000 feet than the heart-wood; while they both seem to be about the same at 7,000 feet. At 9,000 feet, there is an increase in favor of sap-wood, but there is a sudden decrease in the heart-wood. At 10,000 feet, although the value increases in both (in one less than in the other), there hardly seems any difference between the two woods, in this respect. The sponification values, like the percentages of oil and fat synthesis in deodar, do not seem to show any definite tendency. Chemists recognize a relationship between the saponification value and the total fatty acids of an oil. Thus it may be concluded from the figures in column IV, that the manufacturing of total fatty acids in deodar may be independent of the kind of wood or the elevation. Graph No. II shows both the iodine and saponification values in deodar woods grown at various elevations.

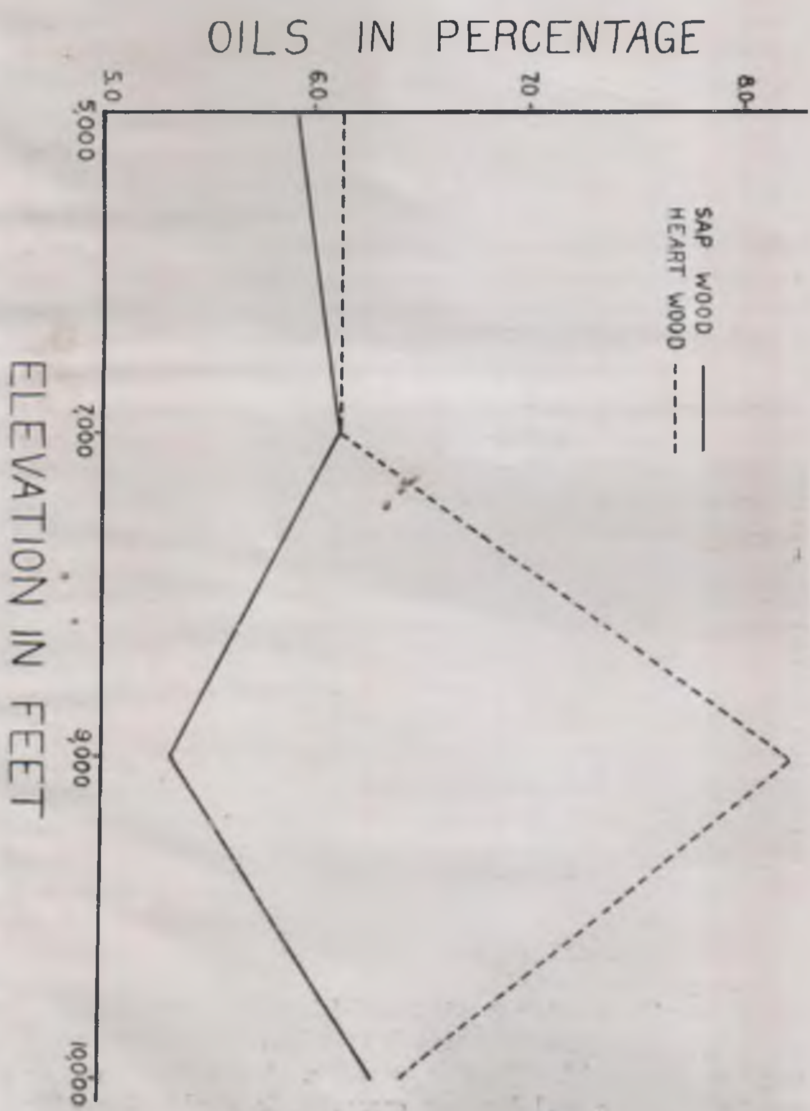
Figures for Reichert's values have been recorded in column V. They do not seem to show any uniformity. For instance, they are about the same at 5,000 feet and 10,000 feet above the sea, but they increase or decrease at 7,000 and 9,000 feet. This trend is irregular both in respect to the elevation and the kind of wood. Variability in Reichert value seems to be reasonable, because the chemists have shown a relationship between this and saponification value of an oil. Since saponification value has not shown any relationship either with the elevation or with the kind of wood, ununiformity in Reichert's value may also be expected.

The last column shows viscosity. There seems a steady but definite increase of time (seconds) for an outflow of heart and sap-wood oils from low to high elevations. Furthermore, the heart-wood oil is less viscous than sap-wood at all elevations except at

10,000 feet, where they are equal. The difference at 7,000 and 9,000 feet are not very pronounced. Even then they can fall within an experimental error. They do show, however, a progressive trend.

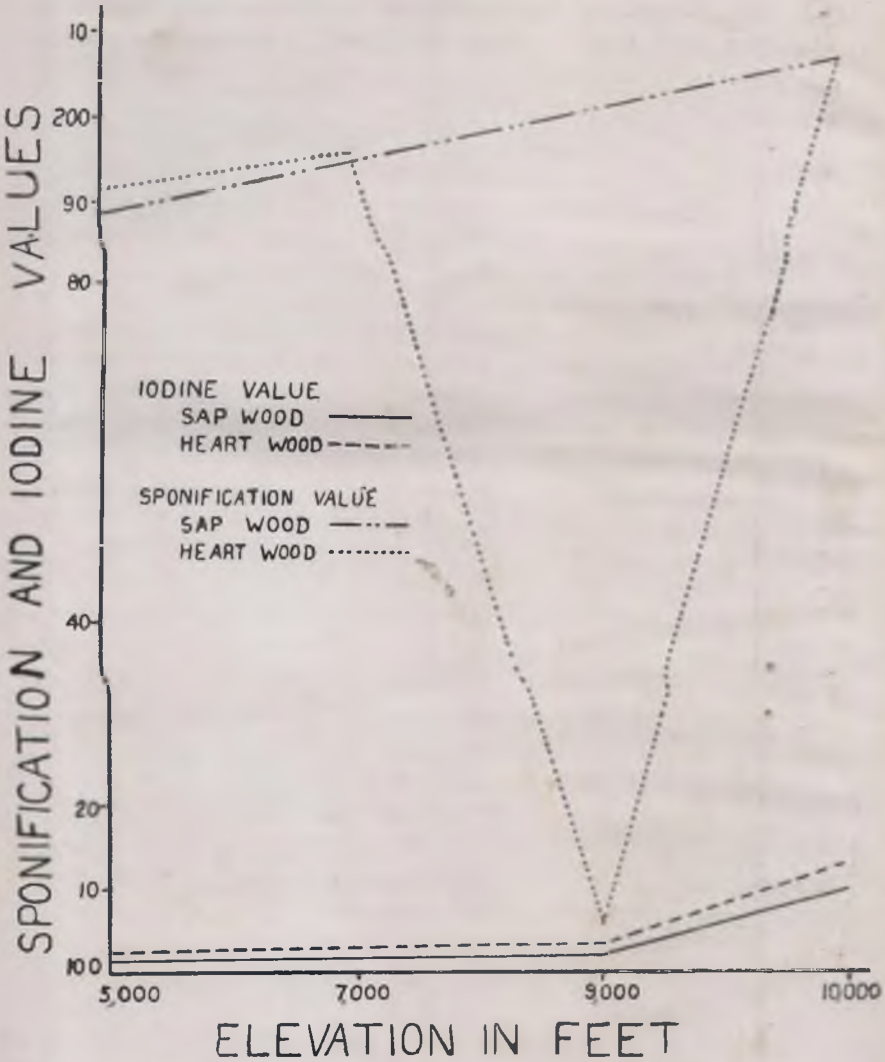
GRAPH No. I.

The Effect of Elevation on the percentages of oils (Ether Extract) found in the Sap and Heart-wood of Cedrus Deodara.



GRAPH No. II.

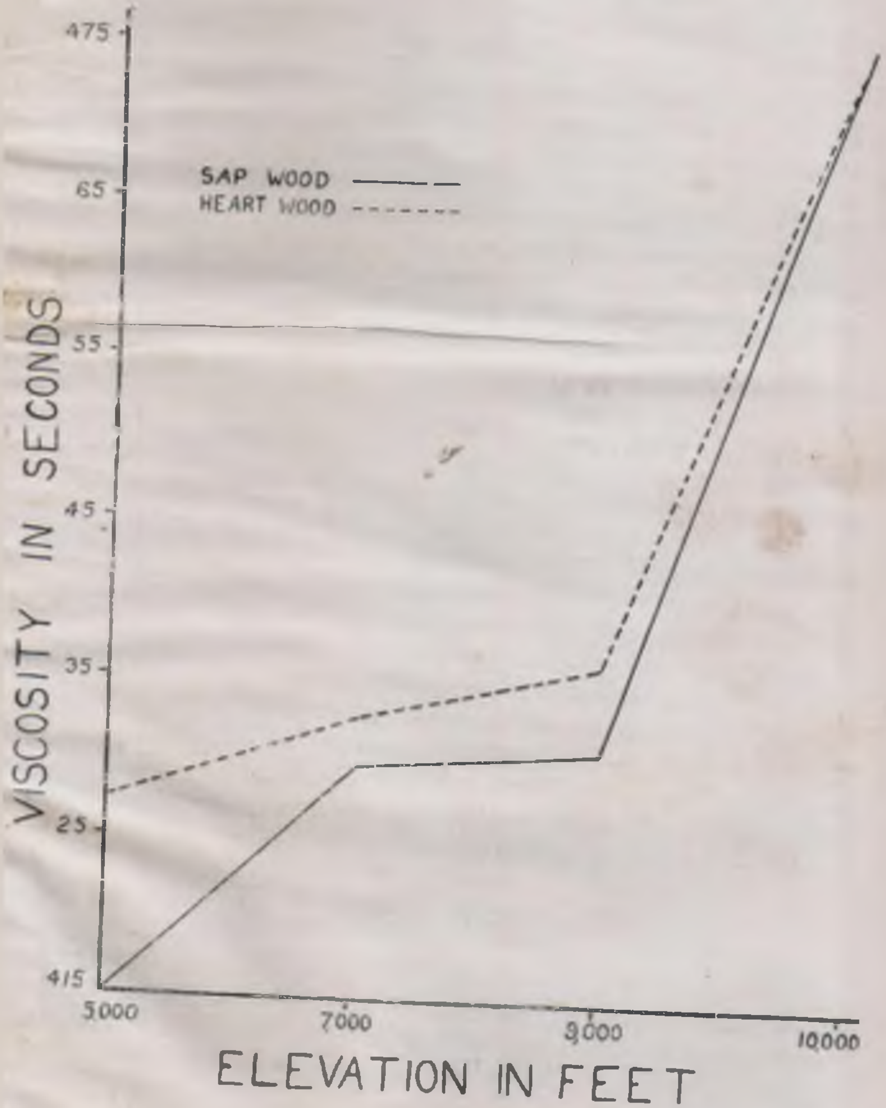
The Effect of Elevation on the Iodine and Saponification Values of Oils (Ether Extract) found in Deodar Woods, Grown at Various Elevations.



More viscous oil in a plant at higher elevations may be an advantage. It may have more caloric value (although not always) or it may form more thick and stable emulsion. It may form a coat over protein molecules and thus may prevent their precipitation. Since some authors have pointed out that winter injury to plants, follows ice formation only, more viscous oils may form colloids, which may prevent or at least slow the water movement to the point of ice

GRAPH No. III.

The Effect of Elevation on the Viscosity (Seconds Required for 50 cc. to Pass through Viscosimeter at 20°c) of Oils (Ether Extract) found in Deodar woods, Grown at Various Elevations.



crystallization. Malhotra (36) has recently shown that sap movement in apple and prune trees depend not only on the area, length or number of trachae but also on the viscosity of the sap itself.

Graph No. III shows viscosity of sap and heart-wood oils at different elevations.

It seems to the writer that these results are not conclusive, because only one kind of tree has been used. However, they do show the necessity of more work to be done in this respect. At present the whole field remains unexplored.

Summary.

1. An attempt has been made to find the quantity of fixed oils and fats in sap and heart-woods of Cedrus Deodara, grown at various elevations of the Himalayas. The data seem to show, that there is no pronounced difference between the percentages of oils in sap and heart-woods due to elevation.

2. Some physical and chemical properties of oils extracted from the heart and the sap-wood have been determined. The results seem to indicate, that on the whole, iodine value is lower for sap-wood than heart-wood. There seems to be some direct relationship between the iodine value and the elevation at which the oil was manufactured.

3. Saponification and Reichert's values for sap and heart-wood oils at various elevations seem to be irregular. The causes of such ununiformity have been pointed out.

4. Viscosity of these oils seems to show a decided relationship between the kind of wood and the elevation (as used in this study). The possible rôle of these changes during death or injury by freezing has been pointed out.

Further experiments are still in progress. Their results will be reported in the next papers of this series.

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