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VARIATIONS IN STARCH & TOTAL POLYSACCHARIDE CONTENT OF PINUS PONDEROSA NEEDLES WITH FLUORIDE FUMIGATION[1]

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INTRODUCTION

Stoklasa (10) reported that leaves of plants showing symptoms of chronic sulfur dioxide injury have a lower starch content than normal leaves. Haselhoff et al (4) suggested that lowered starch content was associated only with chronic sulfur dioxide injury, since, in the instance of acute injury, the starch level is fixed at the level which existed at the time of the cell destruction. This hypothesis was not experimentally substantiated. Swain (11) stated that repeated mild, daily, non-marking sulfur dioxide fumigations resulted in no reduction in carbohydrate content of the leaves. Katz and Pasternack (6) studied the effect of concentrations of sulfur dioxide insufficient to produce leaf markings and found an increase in sulfur content, but no significant change in starch concentration.

No similar studies have been found in the literature relating (a) starch and polysaccharide levels in plant leaves with (b) fluoride fumigation. This work was undertaken to provide information on the relation between (a) starch and polysaccharide content of Pinus Ponderosa needles and (b) fluoride exposure level and sequence of fumigation.

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EXPERIMENTAL

FLUORIDE FUMIGATION PROCEDURES: Five groups of ponderosa pine (*Pinus ponderosa* Laws) were

used, each consisting of six to ten separate trees ranging from four to eight years of age. The trees were grown in 5 gallon cans and fumigated in plastic greenhouses under conditions which have been reported (1).

Group I was the control set and was divided into three sub-groups. Subgroup A was placed in the control greenhouse 8 hours per day, 5 days per week; Subgroup B was placed in the control house 8 hours per day, 2 days per week; and Subgroup C was placed in the control house 4 hours per day, 2 days per week. Groups II and III were fumigated 8 hours per day, 5 times weekly, at 0.5 and 1.5 μg F^-/cu m, respectively. Group IV was fumigated 8 hours per day, twice weekly, at 5 μg F^-/cu m. Group V was fumigated 4 hours per day, twice weekly, at 10 μg F^-/cu m. Trees were maintained out-of-doors when not in one of the greenhouses.

REAGENTS: Perchloric Acid: 72 %, reagent grade. Iodine—potassium iodine: Grind 7.5 g iodine and 7.5 g potassium iodide with 150 ml of water, dilute to 250 ml, and filter through no. 3 Whatman paper with suction. Potassium iodide: 0.4 N. Dissolve 66.408 g of potassium iodide in distilled water and dilute to 1,000 ml. Potassium iodate: 0.005 N. Dissolve 1.07 g of potassium iodate in distilled water and dilute to 1,000 ml. Alcoholic sodium hydroxide: Dilute 350 ml ethanol, 100 ml water, and 25 ml 5 N sodium hydroxide to 500 ml of water and filter. Anthrone solution: Dissolve 1 g of anthrone in 500 ml of ethyl acetate (7). Sulfuric acid: 80 %. Dilute 400 ml sulfuric acid 98 % to 500 ml with water.

ANALYTICAL PROCEDURES: *I. Sample Preparation*: Composite samples of approximately 30 grams each of the current year's needles were obtained weekly from each group of fumigated and control ponderosa pine trees. All samples were immediately dried for 24 hours at 65° C in a forced air oven. The dry needles were ground in a Waring blendor and stored for analysis.

II. Extraction of sugars: A 2 g sample of the finely ground, air dried pine needles was placed in a Soxhlet extractor and extracted for 48 hours with 80 % ethyl alcohol to remove the simple sugars (8). The alcoholic solution was decanted and discarded. The sample was then extracted for 3 hours with ether to complete the removal of alcohol. The sample was air dried and the per cent extractable material present in the original sample was determined by weight difference.

III. Extraction of Acid—hydrolyzable Polysaccharides (starch): One-half gram of dried, alcohol-extracted solids was weighed into a 150 ml beaker and 8 ml of distilled water added. The mixture was heated to boiling and then cooled. The beaker was placed in an ice-water bath and 6 ml of 72 % perchloric acid was added, stirring constantly. The extraction was continued for 1 hour with occasional stirring. The extract was vacuum-filtered through a sintered glass funnel, collecting the filtrate. The residue was washed several times with water, and the filtrate and washings were combined. The extraction was repeated, using 6 ml of water and 4 ml of perchloric water. All filtrates and washing were then combined (8).

IV. Determination of Starch: Forty-three ml of the perchloric acid extract were transferred to a 50 ml volumetric flask. Two ml of 0.4 N KI and 5 ml of 0.0005 N KIO_3 were added and well shaken. The absorbance of the blue color was determined with a spectrophotometer at 660 mμ after 15 minutes (8). The absorptivity was converted to mg of starch by reference to the standard curve.

V. Determination of total polysaccharide: The original perchloric acid extract was diluted 1 : 10 with distilled water. Two ml of the diluted extract were placed in a 10 ml volumetric flask and 0.05 ml of 2 % anthrone in ethyl acetate was added. Then 5 ml of 98 % sulfuric acid were added and well shaken. The flask was allowed to stand for 10 minutes. The reaction mixture was cooled to room temperature and diluted to 10 ml with 80 % sulfuric acid. The light absorbance was spectrophotometrically determined at 630 mμ (7, 8). The absorptivity was converted to weight of polysaccharide by reference to the calibration curve.

VI. Preparation of Standard Curves: An aliquot of a perchloric acid extract of a known weight of soluble starch was transferred to a centrifuge tube and 5 ml of 20 % NaCl and 2 ml of the iodine–potassium iodide reagent were added. The solution was well mixed and allowed to stand for at least 20 minutes. The tube was centrifuged and the supernatant decanted with extreme care to avoid loss of precipitate. The precipitate was washed with 5 ml of alcoholic sodium chloride by gently shaking the tube, centrifuging, and decanting the liquid.

The precipitated starch was then converted to starch by adding alcoholic sodium hydroxide to the tube. The tube was gently shaken and tapped until all of the blue color was discharged. A stirring rod must not be used. Aliquots of the resultant solution were used to establish the standard polysaccharide curve with the anthrone reagent and the starch curve by means of the iodine blue color.

VII. Fluoride Analysis: One gram of air-dried and ground pine needles was slurried with CaO and distilled water for fluoride analysis. The slurry was first dried and partially ashed under infra-red heat lamps and finally ashed in a furnace at 600° C for 30 minutes. The ash was then fused with NaOH (9) and distilled from perchloric acid (12). The distillate was titrated by a modified high-salt-thorium nitrate method (2, 13).

RESULTS

Pine needles were sampled and analyzed each week for starch, total polysaccharide, ethanol extractable material, and fluoride content. Unfortunately, it be-

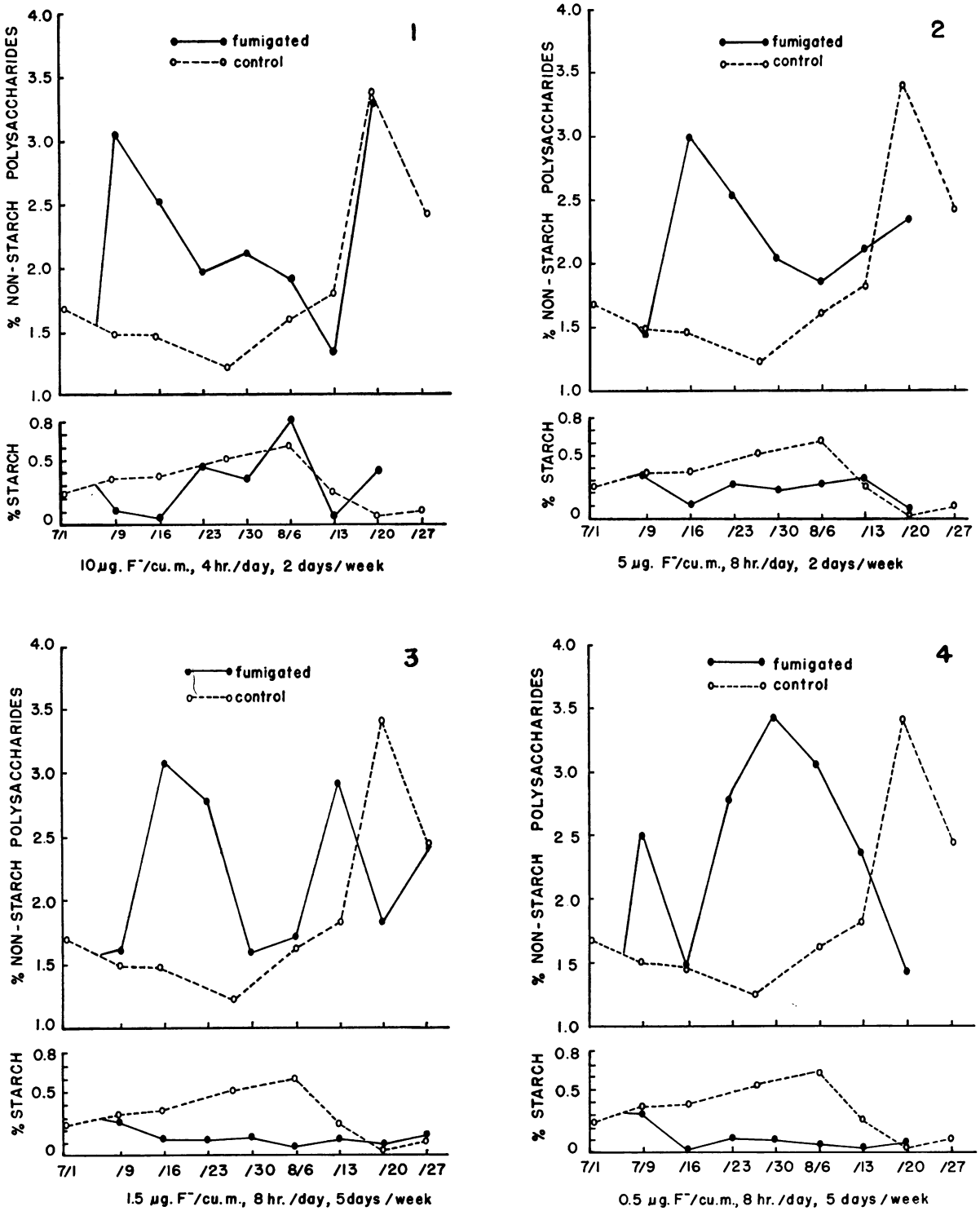

FIG. 1. Starch and non-starch polysaccharide content of fumigated and control pine needles—10 µg F^-/cu m, 4 hr/day, 2 days/week.

FIG. 2. Starch and non-starch polysaccharide content of fumigated and control pine needles—5 µg F^-/cu m, 8 hr/day, 2 days/week.

FIG. 3. Starch and non-starch polysaccharide content of fumigated and control pine needles—1.5 µg F^-/cu m, 8 hr/day, 5 days/week.

FIG. 4. Starch and non-starch polysaccharide content of fumigated and control pine needles—0.5 µg F^-/cu m, 8 hr/day, 5 days/week.

came necessary to discontinue sampling for fluoride analysis following the 3rd week of fumigation so as to reduce the rate of denuding of the test trees. Figures 1 to 4 show the time course of starch and of non-starch polysaccharide content of the ponderosa pine needles fumigated at the four levels of hydrogen fluoride compared with control needles.

These data indicate that A, the concentrations of non-starch polysaccharides were generally inversely proportional to the starch levels in all groups of fumigated trees: B, the starch levels in all fumigated groups declined whereas the non-starch polysaccharides increased rapidly following the initial week's fumigation; C, starch levels in the trees fumigated at the two highest fluoride levels twice weekly equaled or surpassed the starch levels found in the control trees toward the end of the fumigation sequence, whereas the starch levels found in the trees fumigated at the two lowest levels 5 days per week tended to remain at a uniform reduced level throughout the entire period.

Discussion

Comparison of the total fluoride to which each of the four groups of trees was exposed each week may be made using the concept of exposure factor (1). The exposure factor is defined as the arithmetic product of A, the atmospheric concentration of HF, B, the hours per day of exposure and C, the number of days per week of exposure. Thus Group II (0.5 μg F^-/cu m, 8 hours a day, 5 days/week) received an exposure of 20, Group III (1.5 μg F^-/cu m, 8 hours a day, 5 days/week) received an exposure of 60, Group IV (5 μg F^-/cu m, 8 hours a day, 2 days/week) received an exposure of 80, and Group V (10 μg F^-/cu m, 4 hours a day, 2 days/week) received an exposure of 80.

On the basis of the data presented in figures 1 to 4 it appears that one possible action of fluoride upon the polysaccharide system of the pine needles may be to alter the relationship between the non-polysaccharides and starch. The data further indicate the possibility that plants may adapt themselves to concentrations of atmospheric fluorides in the range of 5 to 10 μg F^-/cu m if provided a recovery period between each exposure. Conversely plants may not so readily adapt to lower total, more nearly continuous fluoride fumigations. Similar observations of the relative effects of daily fumigations at the lowest fluoride concentration (1.5 μg F^-/cu m) as compared with the highest concentrations (5 & 10 μg F^-/cu m) on a twice weekly fumigation basis were previously observed when comparing the onset of initial leaf changes at three levels of fluoride concentration (1).

This apparent ability of plants to adapt to or show somewhat greater resistance to intermittent higher fluoride fumigation levels becomes significant in view of recent evidence that such intermittent fluoride fumigations are the type which do in fact exist in the field (3 & Adams & Koppe, 1960, unpubl. information). This is in contrast to the long time, low concentration average atmospheric concentrations (less than 1 μg F^-/cu m) which have previously been assumed to exist by many workers in this field (5).

These data re-emphasize the concept that the sequence of fluoride exposure may outweigh the influence of the actual fluoride level within a fumigation concentration range of 0.5 to 10 μg F^-/cu m.

Summary

Fluoride fumigation of *Pinus ponderosa* Laws resulted in an initial departure of the concentrations of starch and non-starch polysaccharides from similar levels in control tissue. It appeared that one possible mode of fluoride action was to alter the relationship between the non-starch polysaccharides, and starch. The data also indicate that ponderosa pine tend to adapt to fluoride fumigations at higher levels, 5 to 10 μg F^-/cu m, when the exposure is intermittent. This is in contrast to the reaction of the plant to lower fumigation concentrations, 0.5 and 1.5 μg F^-/cu m, of longer and more frequent duration and lower exposure factor. This apparent ability of pine to adapt to somewhat higher but more intermittent fumigation exposures becomes significant in relation to recently obtained information showing that intermittent fumigations do exist in the field (3). It had previously been assumed that field fumigations were of the long-time, low average concentration type (5).

The variation-range for the concentration of starch and for that of non-starch polysaccharide was not statistically different for the fluoride-fumigated plants and in the controls. Thus foliage analysis for starch and total polysaccharide levels could not be used to determine whether any particular field-grown plant had been exposed to a fluoride fumigation.

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INFLUENCE OF REPRODUCTIVE ORGANS ON SECRETION OF SUGARS IN FLOWERS OF STREPTOSOLEN JAMESONII, MIERS[1,2]

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Nectar secretion in nectaries of flowers pollinated by insects or birds generally coincides with pollen maturation, and as a result the nectar forager picks up mature pollen on its body. Foraging insects which have been studied exhibit a considerable degree of species constancy (16), and so the viable pollen of one flower is deposited on stigmas of other flowers of the same species. As the stigma is receptive to pollen at this time, the opportunity for fertilization is provided. Secretion by floral nectaries ceases with fertilization and senescence of the corolla (2). In unpollinated flowers secretion persists for a longer time.

In view of these relationships one might suspect the existence of a coordinating mechanism, possibly hormonal in nature, between the events culminating in pollen maturation and those leading to nectar secretion. An interesting example of an apparent correlation may be observed in nasturtium, (Tropaeolum sp.), in which part of the corolla forms a spur containing the nectary. Prior to pollen maturation, the filaments are curved away from the side of the flower which carries the spur. As the anthers dehisce, the filaments straighten so that their anthers are directly above the mouth of the spur. Nectar secretion begins at this time. Foragers seeking nectar in the spur, of necessity come into contact with the anthers and pick up mature pollen.

In a study of nectar secretion in plants with unisexual flowers, Fahn (4) found considerable differences between the nectar yields of staminate and pistillate flowers. The sex of the higher yielding flowers varied with the species. These observations suggested a relationship between the stamens and pistils and nectar secretion. The present work relates to the influence of the anthers and stigma on secretion in floral nectaries of *Streptosolen jamesonii,* Miers. In this species the flowers are perfect and the nectary forms part of the ovary. As nectaries and stigmas of some species have structural and functional features in common (3), the nectary and stigma of Streptosolen were compared with respect to their anatomy and secretory activity.

In addition to the outward movement of sugar in nectar secretion, the reverse movement was examined. Since Bonnier's suggestion (2) that nectar is reabsorbed by the flower after fertilization, indirect evidence of reabsorption, based on slight increases in total nectar yield with periodic removal of nectar (15) has been obtained. Recently Pedersen et al (14) have provided direct evidence of reabsorption by supplying C^{14}-labelled sucrose to pollinated alfalfa florets. Radioactivity was found throughout the plant. Absorption was not tested in unpollinated flowers. Ziegler and Lüttge (24) have demonstrated absorption of C^{14}-labelled glutamic acid by secreting nectaries of *Daucus carota, Abutilon striatum,* and *Viburnum opulus.*

Materials & Methods

Plant Material. *Streptosolen jamesonii* Miers is a shrub of the Solanaceae family which flowers profusely over several weeks and produces nectar in abundance. Nectar secretion commences around the time the flower opens and continues for about three

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