Studies on Auxins and Growth Inhibitors in Japanese Red Pine (*Pinus densiflora*)

Ryuzo OGASAWARA*

Introduction

Auxin is an indispensable substance for plants, namely this auxin is essential to growth and differention of organs ··· buds, leaves, flowers, roots, and so on.

Many workers have investigated the chemical nature and the function of auxin, and there has been great achievment in this field.

Our knowledge in this field about pine, however, is still too limited.

Hitherto, investigation of the pine auxin has been carried out by a few workers, for example, Czaja[6] in *Pinus silvestris* and *Pinus Hedreichii*, Zimmermann[30] in *Pinus strobus*, Mirov[13] in ponderosa pine and torrey pine, Onaka[20] in *Pinus Thumbergii*, Fransson[8] in *Pinus silvestris*.

But, the chemical nature of pine auxin unfortunately, is not confirmed in detail.

As is well known, the growth inhibitor is produced in many plants.

It is assumed that this growth inhibitor is a substance which, being produced in a particular part of the plant and influences a specific physiological process.

The chemical nature of pine growth inhibitor and their in vivo function are not yet established.

It is a plaesure to acknowledge the valuable advice of Prof. Y. KONDO given to the writer during this experiment.

Materials and Methods

4-year-old red pine (*Pinus densiflora*) was used as experimental materials.

1. Extraction of auxin and growth inhibitor

i. Ether extraction

20 g sample was taken from the pine (buds, leaves or roots), cut into slices after freezing and was extracted at 2 degrees C with 150 ml of ether for 20 hours in the dark.

This ether extract was shaken repeatedly with 2% of sodium bicarbonate solution. Ether extract was evaporated to a small volume (neutral fraction).

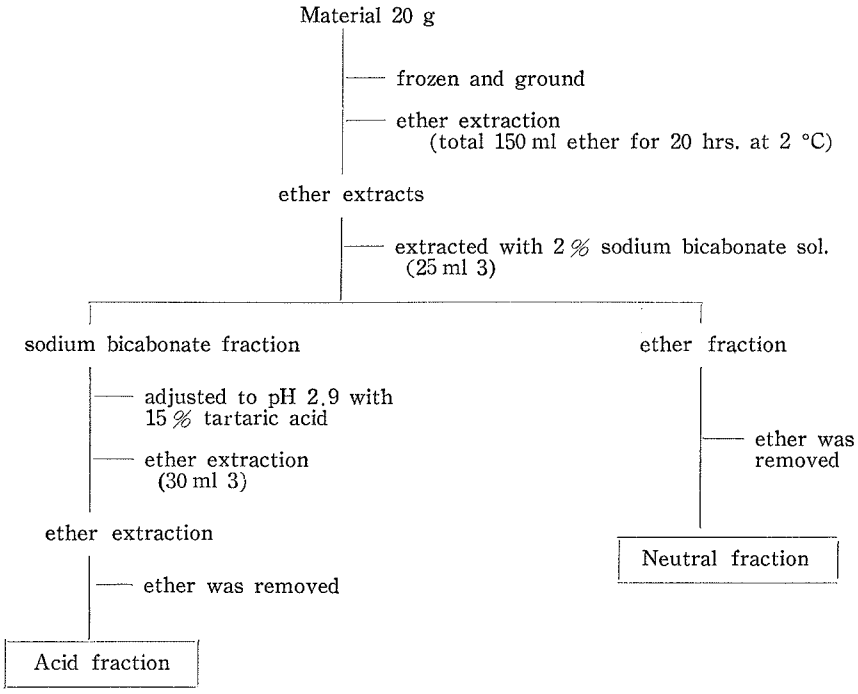
The aqueous fraction was adjusted to pH 3.0 with 15 % tartaric acid solution and then the solution was extracted with ether. This ether was evaporated to a small volume (acid fraction).

This method is sumarized in Table 1.

2. Water treatment

* Lab. of Silviculture, Fac. of Agr., Tottori Univ., Tottori

Table 1 Method of extracting auxin and inhibitor from buds, roots and leaves of pine



a. 200 g fresh sample (beds and leaves) was soaked in 200 ml of hot water (80~100 °C) for 10 minutes.

The water extract was filtered through filter paper (No. 2 Toyo Roshi Co.) and the filtrate was shaken four times with 100 ml of ether.

b. 20 g fresh sample was soaked in some volume water at 25°C for 24 hours.

Next, auxin and growth inhibitor in this ether extract (a) and in this treated sample (b) were extracted with the same method as that described in ether extraction, only the amounts being different.

Ascending chromatography on Tokyo No.50 filter paper was performed.

2. Paper chromatography

The technique of paper chromatography was used for the identification and purification of the extracts.

The residual substances obtained by ether evaporation were used for the analysis. This chromatogram was developed in the solvent for about 20 cm in a glass cylinder at room temperature.

The solvents are as follews ;

1. isopropanol - 28 % ammonia - water (8 : 1 : 1, in volume)
2. butanol - ethanol - water (4 : 1 : 1, in volume)
3. butanol - ethanol - 28 % ammonia (1 : 1 : 2, in volume)
4. 70 % ethanol

3. Avena straight growth test

The paper developed in isopropnol-ammonia-water (8 : 1 : 1) was dried and then it was cut transversally into 10 segments.

This paper segment was immersed in 2ml of 2% sucrose solution in a small Petri dish at 2 °C in the dark and after 20 hours it was removed.

Avena seedlings (Victory No.1) were grown at 25 °C in the dark, and when the height of seedlings reached 2.5~3.0 cm, the tips of the coleoptiles were decapitated.

10 sections of 2.3 mm long coleoptiles (from 3 mm below the tip to 5.3 mm) were placed in this Petri dish. After incubation at 25 °C in the dark for 20 hours, the length of Avena sections was measured.

As control only th length of these sections was measured which were immersed in the solution which the unspotted control chromatogram paper

4. Color reaction

The paper chromatogram developed in solvent was dried.

This chromatogram was sprayed by reagent and then was heated for a few minutes thermostatically controlled at 60~70 °C for color development.

Reagents are as follows ;

1. Ehrlich reagent (p-dimethylaminobenzaldhyde 2g-20 ml HCl-80ml abs. ethanol)
2. Gordon & Weber reagent (0.05 M $FeCl_3$-5 % $HClO_4$, 1 : 50 in volume)
3. Mitchell & Brunstetter reagent (KNO_2-HNO_3, 1 g-200 ml)
4. Tang &Bonner reagent (0.5 M $FeCl_3$-H_2SO_4-H_2O, 3 : 60 : 100 in volume)

Tests on the inhibiting action of Salkowski's color reaction of IAA were carried out with Shibaoka's method.[21]

5. The treatment of tryptophane

20 g of sample was treated with 1000 ppm solution of DL-tryptophane at 25 °C for 24 hours in the dark.

After the treatment, the auxins and growth inhibitors in the sample were measured with the above described method.

The following abbreviations are used in this paper.

IAA……indole-3-acetic acid

IAN……indoleacetonitrile

Results

Chromatograms of ether extracts from buds, leaves and roots are illustrated in Fig. 1~3.

With chromatographying in isopropanol - ammonia - water (8 : 1 : 1) one growth promoting zone (Rf around 0.00~0.50 in acid fraction) and two inhibiting zones (Rf around 0.50~1.00 in acid fraction and Rf around 0.40~1.00 in neutral fraction) were detected.

Chromatography reveals a growth promoting substance which corresponds in Rf with

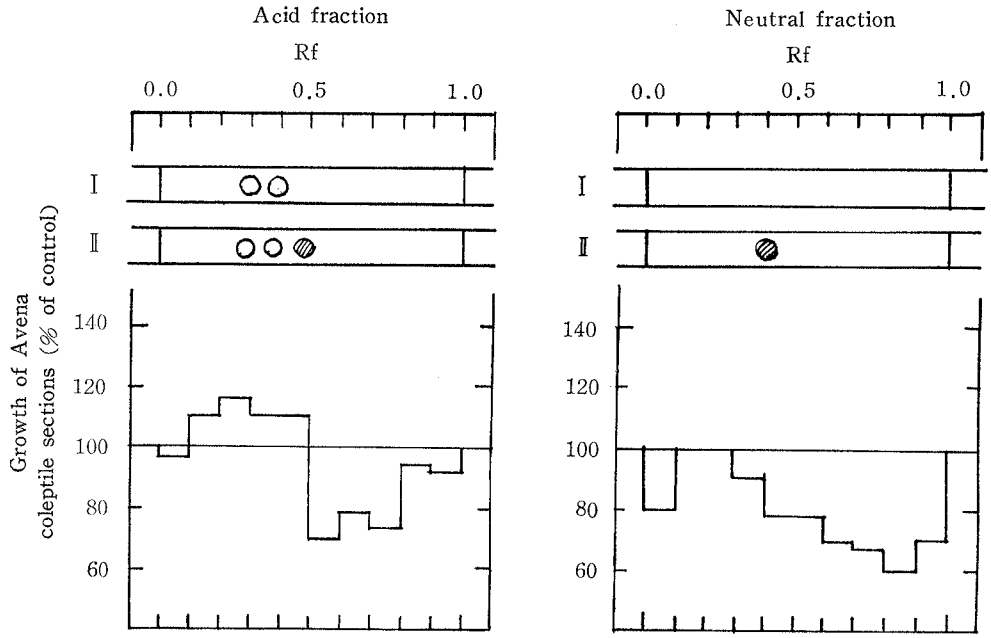


Fig. 1 Chromatograms of ether extract obtained from buds, developed in isopropanol-ammonia-water (8:1:1), assayed by Avena straight growth test.
I : Reactions of chromatograms by Ehrlich reagent (lst of April).
II : Reaction of guide chromatograms of adding synthesized IAA to ether extract by Ehrlich reagent.

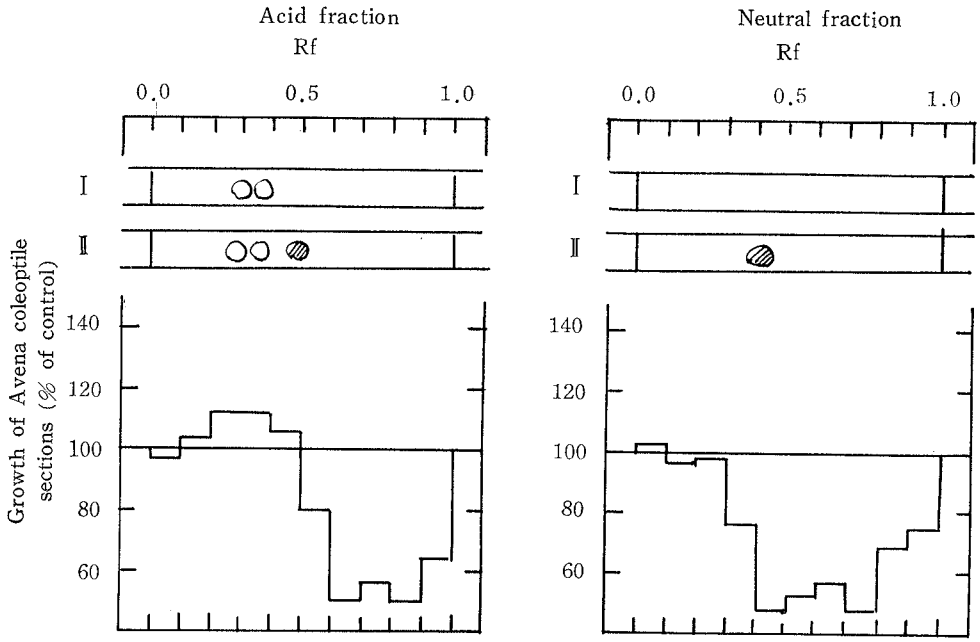


Fig. 2 Chromatogram of ether extracts obtained from leaves, assayed by Avena straight growth test (lst of April).
I : Color reaction of chromatogram by Ehrlich reagent.
II : Color reaction of guide chromatogram of adding synthesized IAA to ether extract by Ehrlich reagent.

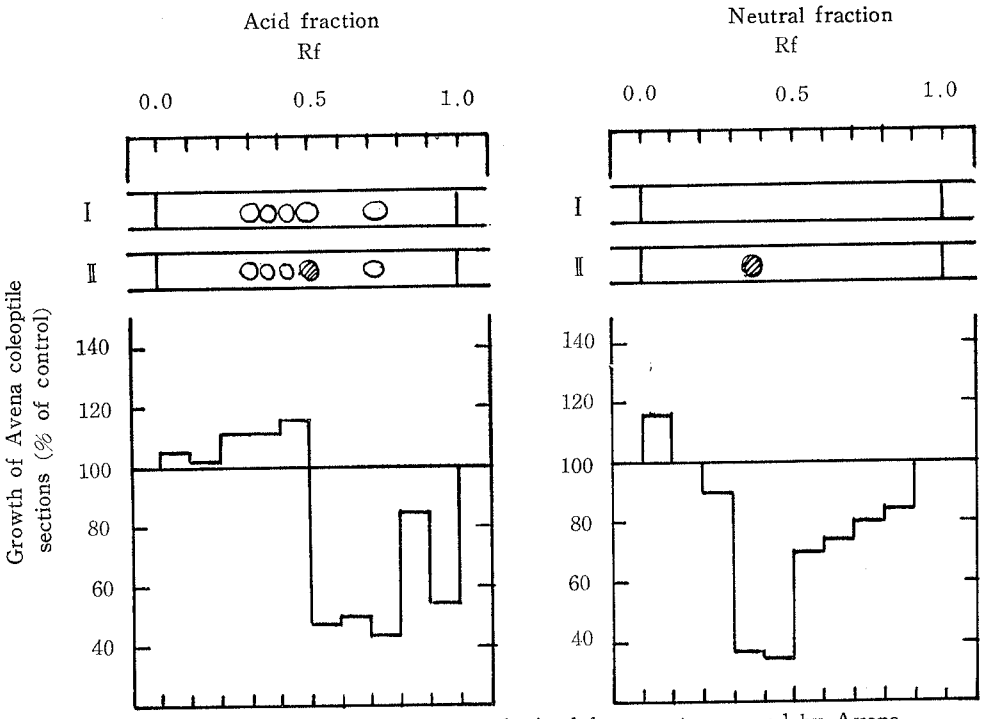


Fig. 3 Chromatogram of ether extract obtained from roots, assayed by Avena straight growth test (lst of April).
I : Color reaction of chromatogram by Ehrlich reagent.
II : Color reaction of guide chromatogram of adding synthesized IAA to ether extract by Ehrlich reagent.

IAA. The other chromatogram paper, which was developed at the same time, was sprayed by Ehrlich reagent.

Two or four substances (A : Rf around 0.28, B : Rf around 0.34, C : Rf around 0.38 and D : Rf around 0.48) in growth promoting zone were found.

Color reaction and Rf value of these substances are presented in Table 2. Among

Table 2 Color reaction and Rf value of substances in growth promoting zone of ether extract.

Substance	Rf value			Color reaction		
	Isopropanol -ammonia -water	Butanol -ethanol -ammonia	70% ethanol	Ehrlich	Gordon & Weber	Tang & Bonner
Substance A	0.28	0.45		Green	Pink	Pink
Substance B	0.34	0.57	0.64	Blue	Purple	Pink
Substance C*	0.38			Pink		
Substance D*	0.48	0.74	0.80	Blue	Pink	Pink
Synthesized IAA	0.47	0.75	0.80	Purple	Pink	Pink

* These substances were detected only in roots.

them, color reaction and Rf value of substance D were similar to that of synthesized IAA.

But this substance D was observed in roots, while it was not seen in buds and leaves.

Chromatograms extract obtained of hot water from the shoots are illustrated in Fig. 4. On occasions, three substances (Rf around 0.63 and Rf around 0.83 in acid fraction, and Rf around 0.75 in neutral fraction) showing positive reaction by Ehrlich

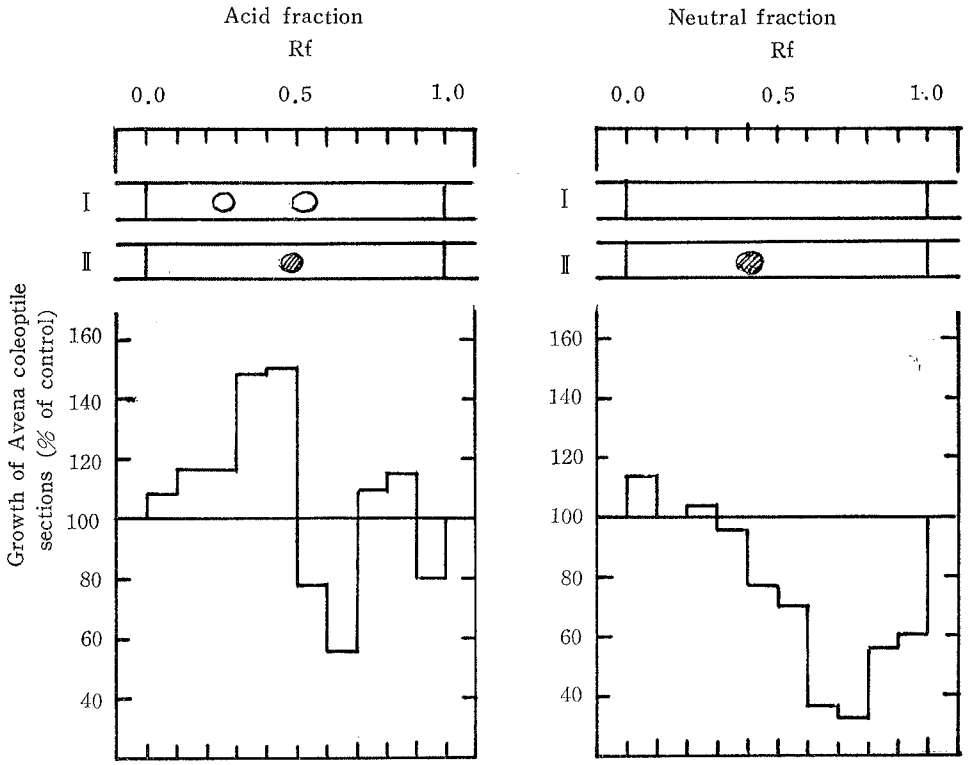


Fig. 4 Chromatogram of hot water extract obtained from buds and leaves, developed in isopropanol-ammonia-water (8:1:1), assayed by Avena straight growth test.
I : Color reaction of chromatogram by Ehrlich reagent.
II : Color reaction of guide chromatogram of adding synthesized IAA to hot water extract by Ehrlich reagent.

reagent were detected on inhibiting zones. Color reaction and Rf value on these substances which corresponds in Rf with IAA are presented in Table 3.

Table 3 Color reaction and Rf value of substance which corresponds in Rf with IAA, in hot water extract from buds and leaves.

Substance	Rf value			Color reaction		
	Isopropanol -ammonia -water	Butanol -ethanol -ammonia	70% ethanol	Ehrlich	Gordon & Weder	Targ & Bonner
Substancd in extract	0.49	0.72	0.64	Bluc	Pink ?	Pink ?
Synthesized IAA	0.46	0.73	0.66	Purple	Pink	Pink

Result on substance in extract was similar to that of IAA.

Chromatograms of ether extract obtained from buds treated with water are illustrated in Fig. 5. Color raction of a growth promoting substance which

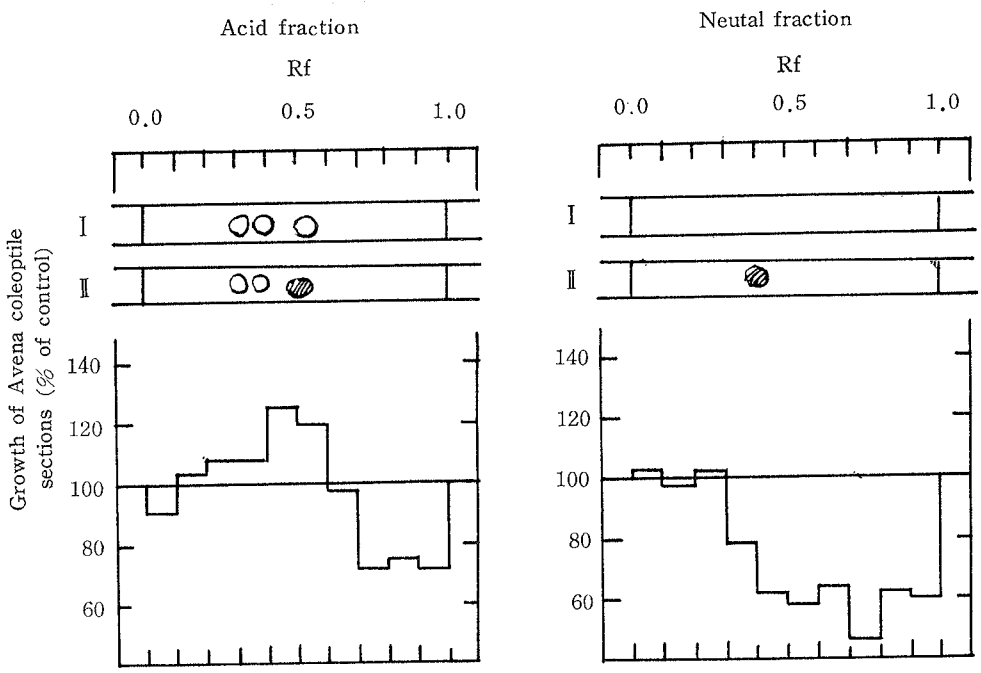


Fig. 5 Chromatogram of ether extract obtained from buds were soaked in water at 25° C for 24 hours, assayed by Avena straght growth test.
I : Color reaction ot chromatogram by Ehrlich reagent.
II : Color reaction of guide chromatogram of adding synthesized IAA to ether extract by Ehrlich reagent.

corresponds in Rf with IAA was similar to that of IAA.

But this similar substance as IAA was not alway found every time.

It is generally considered that IAA is produced from tryptophane by the action of the enzyme.

Chromatograms of ether extracts from buds and roots, which were collected at the same as in the above expriments (Fig. 1~3) and were treated with tryptophane, are illustrated in Figs. 6~7.

The substance which corresponds in Rf with IAA increased and color reaction of this substance was similar to that of synthesized IAA as shown Table 4. This result indicated that red pine has a faculty of IAA production from tryptohane.

Growth inhibitors found in red pine were compared in respect of color reaction and Rf value with synthesized growth inhibitors as shown in Table 5.

The same growth inhibitors found in pine could not be found in synthesized substances.

As shown in Fig. 1~3 and 8, auxins and growth inhibitors distributed throughout

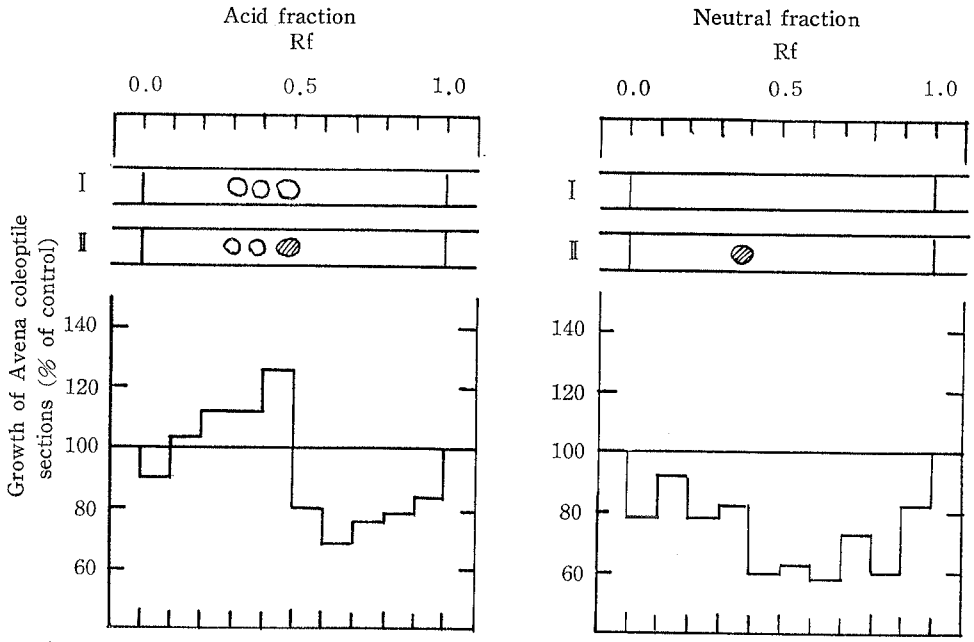


Fig 6 Chromatogram of ether extract obtained from buds were treated with 1000 ppm solution of tryptophane, assayed by Avena straight growth test. (lst of April)

I : Color reaction of chromatogram by Ehrlich reagent.
II : Color reaction of guids chromatogram of adding synthesized IAA to ether extract by Ehrlich reagent.

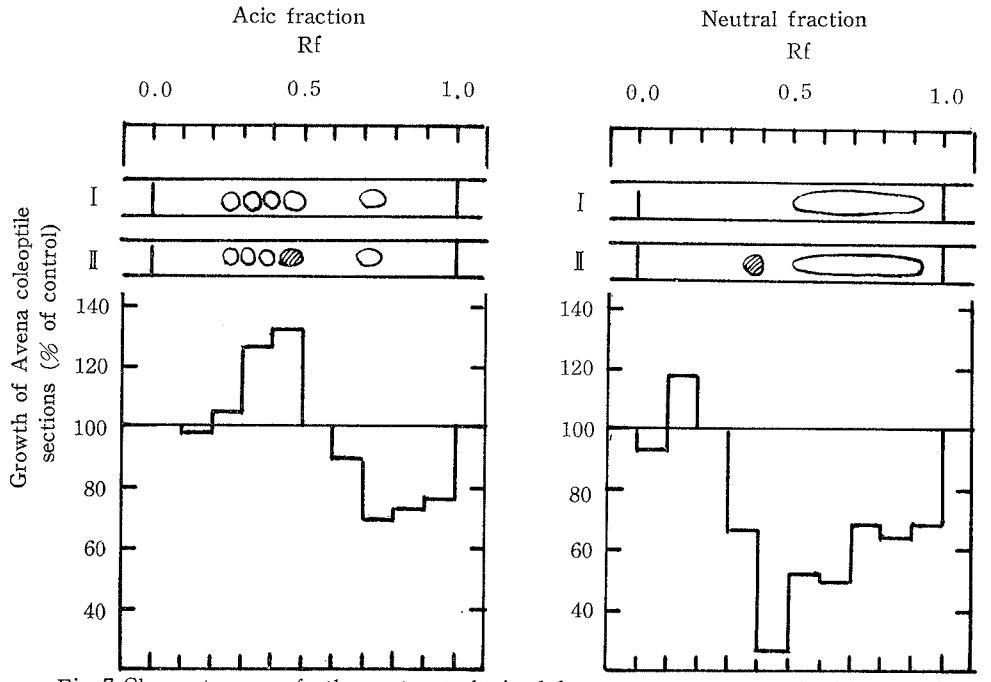


Fig 7 Chromatogram of ether extract obtained from roots were treated with tryptohane, assayed by Avena straight growth test. (lst of April)

I : Color reaction of chromatogram by Ehrlich reagent.
II : Color reaction of guide chromatogram of adding synthesized IAA to ether extract by Ehrlich reagent,

Table 4 Color reaction and Rf value of substance which corresponds in Rf with IAA, in ether extract obtained from buds and leaves treated with tryptophane.

Substance	Rf value		Color reaction	
	Isopropanol -ammonia -water	70 % ethanol	Ehrlich	Gordon & Weber
Substance in extract	0.45	0.73	Purple	Pink
Synthesized IAA	0.45	0.74	Purple	Pink

Table 5 Color reaction and Rf value of growth inhibitors.

Ether extract

Substance	Rf	Ehrlich	Gordon & Weber	Tang & Bonner	2 % $FeCl_3$	Inhibiting action in Salkowski's color reaction of IAA	Note
Sustance F	0.80	Pink	—	—	—	None	Acid
Acid inhibiting zone	0.50～1.00	—	—	—	—	None	except SubstanceF
Neutral inhibiting zone	0.40～1.00	—	—	—	—	〃	
Substance G*	0.63	Pink	Blue	Blue		None	Acid
Substance H*	0.83	Pink	—	—	—	〃	Acid
Substance I*	0.75	Pink	—	—	—	〃	Neutral
Salicylic acid	0.60	?	?	—	Purple	〃	Acid
Cinnamic acid	0.54	—	—	—	Yellow ?	〃	〃
Cumaric acid	0.27	—	—	Purplish grey ?	Orange	〃	〃
Quercetin	?	Light Pink ?	—	—	Yellowish grey ?	〃	〃
Naringenine	0.42	—	—	—	Purplish brown	〃	〃

* These substances were found occasionally in hot water extract.

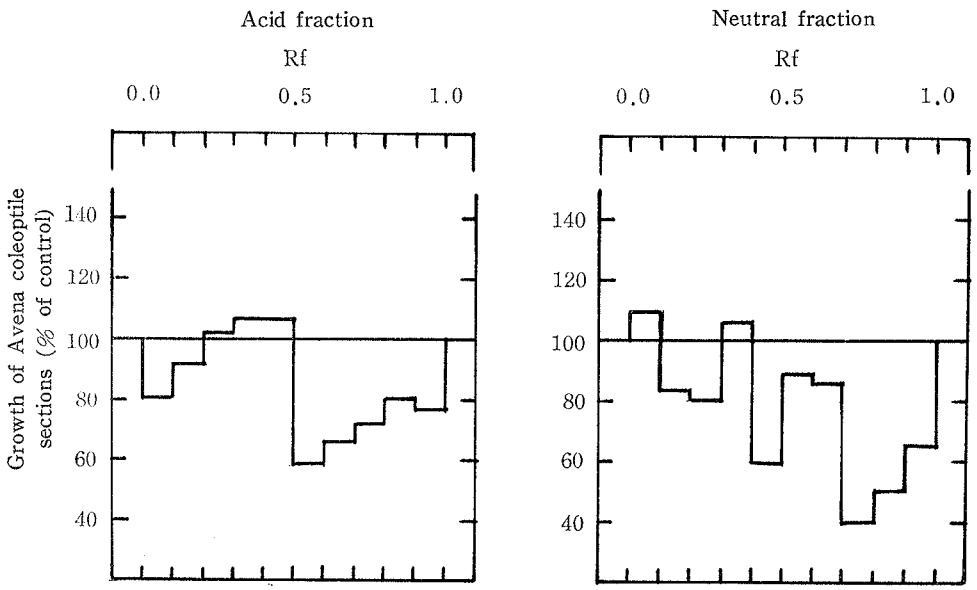


Fig 8 Chromatogram of ether extract obtained from stem, developed in isopropanol-ammonia-water (8:1:1), assayed by Avena straight growth test. (lst of April)

various parts of pine and the concentration of auxins falls off with increasing distance from the tip and rises again in roots.

The experimental results in seasonal variation of auxins and growth inhibitors are illustrated in Fig. 9.

Auxins were detected in April–October and they decreased in December–February. Growth inhibitors were present in all seasons, but, their concentration in winter is more than in other seasons.

Discussion

At present, IAA, IAN and a few other indole compounds are known as natural auxin. Among them, IAA exsits most commonly in vegatable kingdom.

Our knowlege on pine auxin is limited.

Czaja[6] reported on the quantity of auxin in shooting buds of *Pinus silvestris* and *Pinus Hedreichii*. Also, Zimmermann[30] described the quantity of auxin in buds of *Pinus strobus* compared with other trees.

Mirov[13] investigated the distribution of auxin, its movement and the relation between auxin and radial growth etc. in Ponderosa pine and Torrey pine. A similar experiment was carried out using *Pinus Thunbergii* by Onaka[20].

None of these investigators, however, touched on the chemical nature of pine auxin.

Frasson[8] reported that activity, diffusion etc. of auxin present in *Pinus silvestris* differ from that of pure IAA. From this result, he concluded that pine auxin is not

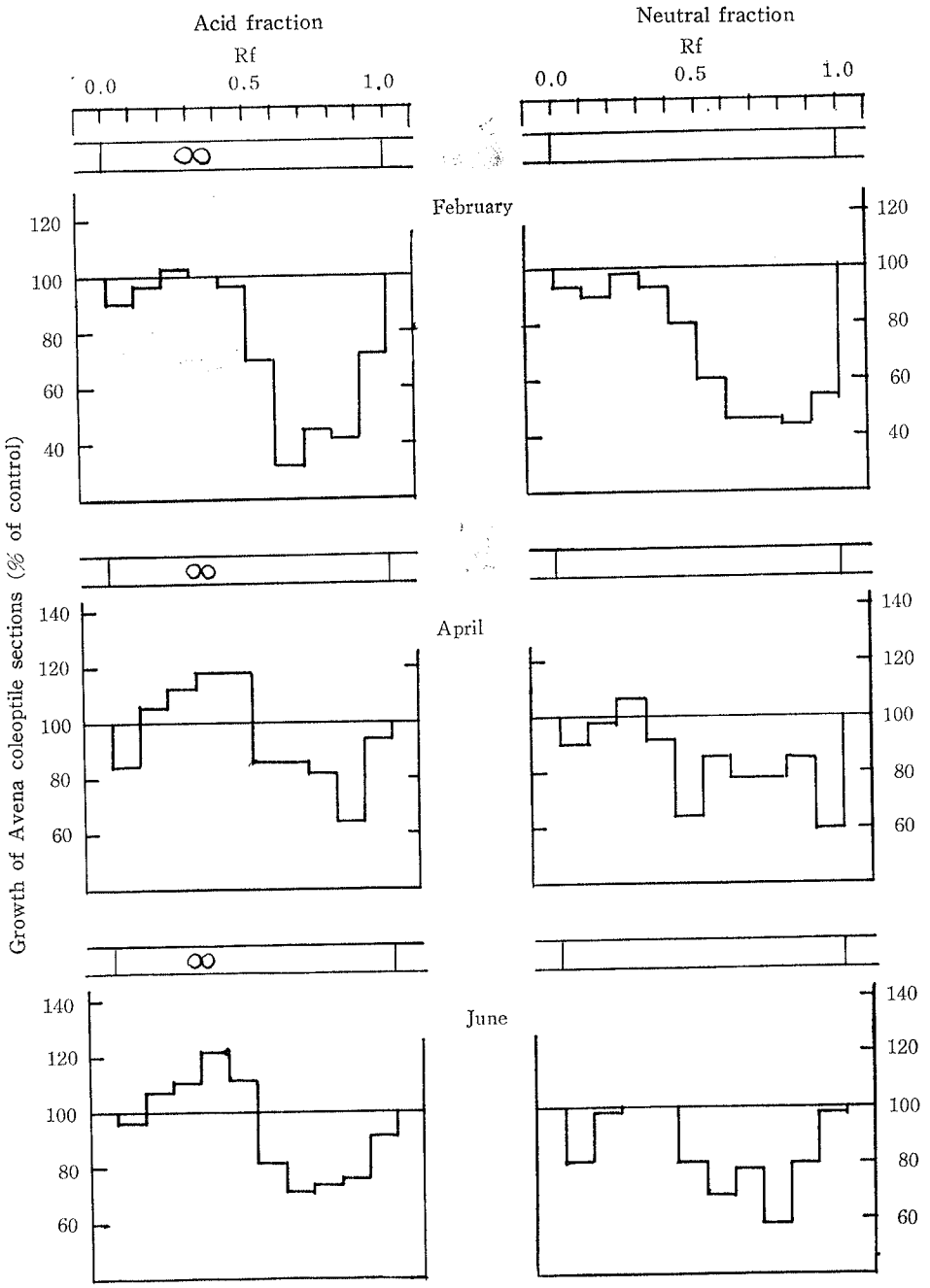


Fig.9(a) Seasonal variation of auxins and growth inhibitors in ether extract obtained from shoots.

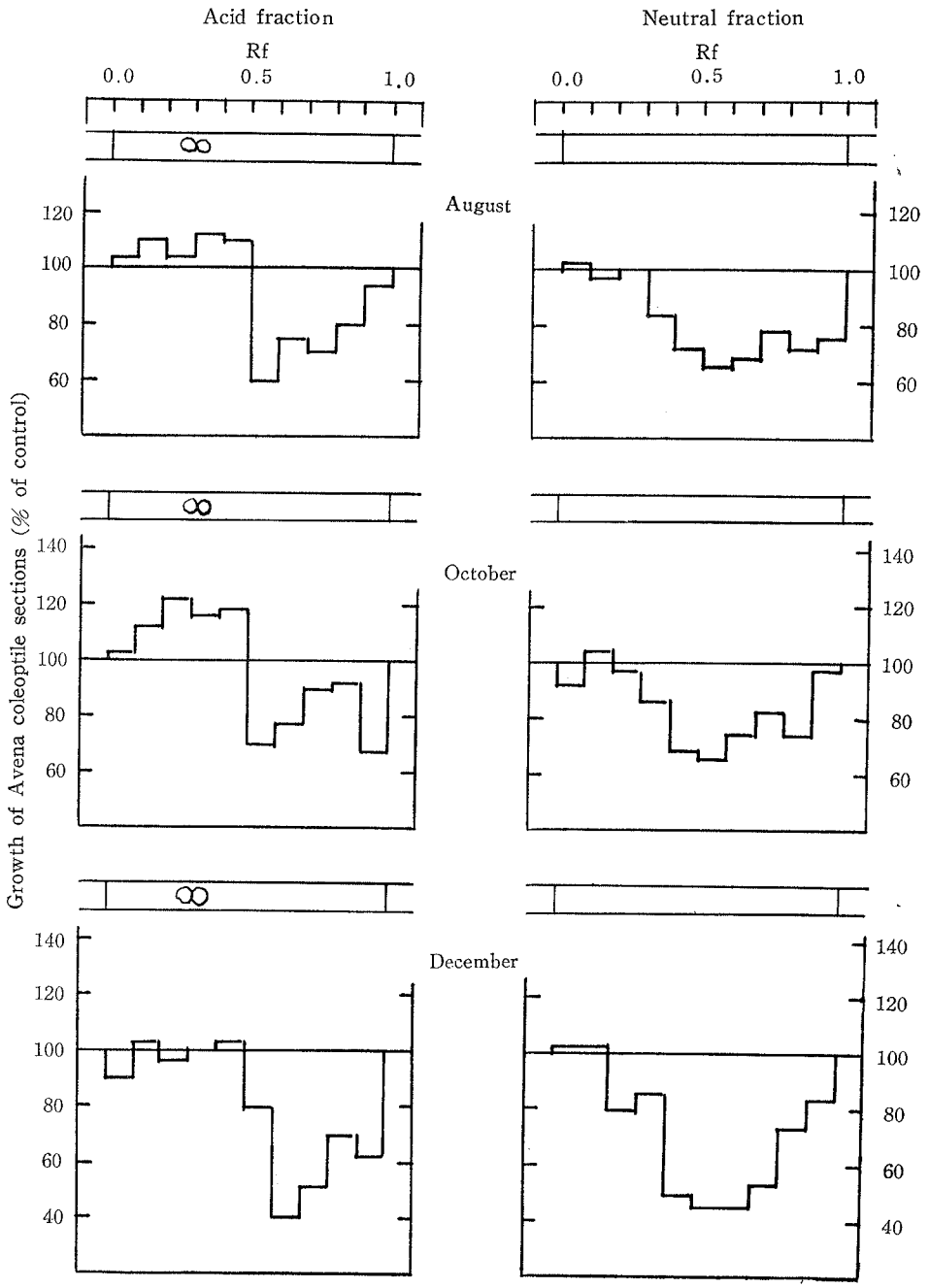


Fig. 9(b) Seasonal variation of auxins and growth inhibitors.

IAA but is something else.

Mirov and Stanley[14] stated that the recent discovery of many auxin-substances or precursors such as indoleacetonitrile and indoleacetoaldehyde indicates the need for reevaluating these older studies of growth substances in pine.

Ogasawara found a few auxins respectively in *Pinus Thunbergii*[16,19] and *Pinus*

strobus[17] and suggested that one of them is IAA.

In *Pinus densiflora*, four substances showing positive reaction by Ehrlich reagent were detected in growth promoting zone.

One of then in roots was identified roughly as IAA by chromatographical analysis.

It may be considered that auxin which corresponds in Rf with IAA in buds and leaves of Japanese red pine is IAA or the similar auxin as IAA, judging from the color reaction and Rf value.

The correct theory upon IAA biogenesis is not yet established.

Thimann[25] stated that at this stage we should still not know for certain whether IAA normally comes from tryptophane, tryptamin, indoleacetonitrile, or some other precursor, or is more directly synthesized.

Setting aside the detailed route, it may be most commonly considered that IAA is produced from tryptophane by the action of the enzyme.

Wildmann et al[28]. separated the enzyme preparation which is capable of converting tryptophan into IAA from spinach leaves.

Moreover, Wildmann and Bonner[29] stated that such an enzyme is distributed through the Avena coleoptile in a manner strikingly paralled to the distribution of auxin itself. Bonner[5] described that tryptophane-IAA converiting enzyme exists in spinach leaves, tobacco leaves, sunflower stem and etc.

If pine produces IAA, it ought to have tryptophane-IAA converting enzyme.

Accordingly, by means of the addition of tryptophane to pine this pine must produce IAA from tryptophane by enzyme.

Japanese red pine has had a faculty of production of IAA from tryptophane.

From this result, it is highly probable that pine produces IAA from tryptophane even without the addition of tryptophane.

Auxin which corresponds in Rf with IAA in ether extract from buds and leaves hardly shows a positive reaction by Ehrlich reagent etc..

It may be suggested that the reason for this is that the quantity of IAA obtained from buds and leaves might be too small to show the positive reaction by reagent, or color reaction is inhibited by pigment.

The reaction of roots by the addition of tryptophane was different from that of buds or leaves.

It is well known that pine roots have the symbiosis with mycorrhizal fungi.

MacDougal and Dufrency[12] fouud auxin to be abundant in mycorrhizal fungi and assumed that this is translocated into the roots and responsible for the coralloid branching of short roots. Slankis[22] provided evidence for this assumption.

Accordingly, it can not be determined whether auxins in Japanese red pine roots were produced by pine itself or by mycorrhizal fungi.

Two substances moving more slowly than IAA detected in red pine are probably indole comounds, judging from their color reaction,

These two substances could be detected throughout the every season, it is probable that these substances are either precursor or weak active auxins.

It is proposed that the formation indolealdehyde which is natural precursor of IAA from tryptophane might occur through two alternative routes.

Tryptophane may be deaminated to indolepyruvic acid and thence decarboxlated to indoleacetaldhyde, or alternatively, tryptophane may be first decarboxlated to tryptamine and then deaminated to indole acetaldehyde.

Stowe and Thimann[23] reported that a spot of lower Rf value than IAA was indentified as indolepyruvic acid in corn kernels.

Nitsch[15] reported that a substance (Rf around 0.32) in strawberry is probably indole pyruvic acid. The above two indole compounds in Japanese red pine are located in the region of indolepyruvic acid.

It may be suggested that one of these substances is indolepyruvic acid.

However, direct comparison with synthesized indolepyruvic acid can not be shown at this time and precise identification of these substances must be deffered.

Thimann[25] stated that prominent among explanations of the control of growth have been the roles assigned to growth inhibitors.

Tagawa[24] stated that the reaction of plant is determined by the algebraical sum total of auxins and growth inhibitors.

Hitherto, many investigators have experimented to establish the function of growth inhibitors in the dormancy.

At present, however, the correct function of growth inhibitors in vivo is not yet established.

The principal reasons for this are considered by Thimann[25] as follows : The growth inhibition is usually not tested on the object assumed to be inhibited, but on sections of oat or wheet coleoptiles, which doubles have a very different susceptibility and even on the test objects employed, little attempt is usually made to relate the extent of inhibition to the amount or concentration of the inhibitor.

Growth inhibitors in Japanese red pine, for the most part, by means of paper chromatography and Avena straight growth test are found in the region of higher Rf value than IAA.

Growth inhibitors in acid fraction are similar to inhibitor-β of Bennet-Clark and Kefford[3] .

It may be considered that red pine growth inhibitors are composed of more than two substances, judging from color reaction.

Davis[7] named growth inhibitor from walnut juglone. Hendershott and Walker[10] reported that growth inhibitor in dormant buds of peach was identified as naringenin

Moreover, the presence of salicylic acid. cumarinic acid, cinnamic acid, quercetin etc. in the vegetable kingdom is well known.

Growth inhibitors in red pine were compared with respect to color reaction and Rf value with a few synthesized growth inhibitors.

The same growth inhibitor found pine could not be found.

But, it seems hardly possible to identify these growth inhibitors only by color reaction and Rf value in this experiment.

The precise identification of pine growth inhibitors must be deferred.

Auxins and growth inhibitors are distributed throughout other part of red pine, and the concentration of auxins tend to fall off with increasing distance from tip (buds) and rise again in roots.

The similar result is well known in some other plants[11,26]

In pine, a seasonal variation of auxins and growth inhibitors has not been sufficiently known.

Onaka[20] reported that in buds of *Pinus Thunbergii*, auxin appeared with the inception of height growth and increased with progress and the maximum was found near the region of the greatest growth, but the amount of auxin did not diminish so much after the elongation ceased and considerable quantity could be detected throughout the growing season.

Allen[1] reported the lowest level of inhibitory substances measured occurred in the period of most rapid elongation.

Auxins of Japanese red pine were detected throughout the growing season (from April to October) and rapidly decreased in the rest period (from December to February). In contrast, growth inhibitors were detected in all seasons but the amount was more in rest period than in growing season.

Hemberg[9] pointed out that the inhibiting substances are related to dormancy of potato tuber.

Allen[1] showed that the acid growth promotors and inhibitors which regulate the winter rest period of longleaf pine buds or are closely connected with regulation of the rest period.

In Japanese red pine, it may be considered that auxins and growth inhibitors are closely correlated with growing season and rest period.

Summary

Auxins and growth inhibitors in buds, leaves and roots of Japanese red pine were investigated by means of paper chromatography followed by bioassay with straight growth of Avena coleoptile section.

On the chromatographying in isopropanol-ammonia-water (8 : 1 : 1), one growth promoting zone (Rf around 0.00~0.50 in acid fraction) and two growth inhibiting zones (Rf around 0.50~1.00 in acid fraction and Rf around 0.40~1.00 in neutral fraction) were detected in April.

Chromatgraphy reveals a growth promoting substance which corresponds in Rf with IAA. This substance showed a positive reaction by Ehrlich reagent etc. in roots, but very rarely in buds and leaves.

It may be suggested that this substance is IAA or the similar substance as IAA

Buds, leaves and roots produced IAA by addition of tryptophane.

From these results, it is probable that red pine produces IAA under normal conditions.

It may be suggested that other two substances showing positive reaction by Gordon & Weber and Tang & Bonner reagents in promoting zone are indole compounds and either precursors or weak active auxins.

It could not be determined whether auxins in roots were produced by pine itself or by mycorrhizal fungi.

A few growth inhibitors exist in pine, but the identification of these substance must be defferred.

Auxins and growth inhibitors distributed throughout other parts of pine and the concentration of auxins tended to fall off with increasing distance from tip and rise again in roots.

Auxins were detected in April-October (growing season) but hardly detected in December (rest period).

Growth inhibitors exist in all seasons and the concentration in rest period was more than in other seasons.

From these results, it may be suggested that auxins and growth inhibitors are closely correlated with growing season and period of Japanese red pine.

References

1. Allen, R.M. : Physiologia Plantarum **13** ; 555~558 1960
2. Audus, L.J. : Plant Growth Substances 1953
3. Bennet-Clark, T.A. and Kefford, N.P. : Nature **171** ; 645~647 1953
4. Blommaert, K.L.J. : Nature **174** ; 970~972 1954
5. Bonner, J. : Plant Biochemistry 1952
6. Czaja, A.Th. : Ber. D. Deut. Bot. Ges. **52** ; 267~271 1934
7. Davis. E.F. : American Jour. Botany **15** ; 620 1928
8. Fransson, P. : Physiologia Plantarum **6** ; 544~549 1953
9. Hemberg, T. : Physiologia Plantarum **5** ; 115~129 1952
10. Hendershott, C.H. and Walker, D.R. : Science **130** ; 798~800 1959
11. Leopold, A.C. : Auxins and Plant Growth 1955
12. MacDougal, D.T. and Duffrenoy, J.M. : Plant Physiology **10** ; 440~465 1944
13. Mirov, N.T. : Jour, Forestry **39** ; 457~464 1941
14. Mirov, N.T. and Stanley, F.C. : Plant Physiology **10** ; 223~238 1959
15. Nitsch, J.P. : Plant Physiology **30** ; 33~39 1955
16. Ogasawara, R. : Jour. Japan Forest Soc. **43** ; 50~54 1961
17. Ogasawara, R. : Jour. Japan Forest Soc. **43** ; 307~310 1961
18. Ogasawara, R. and Kondo, Y. : Trans. Tottori Soc. Agr. Sci. **14** ; 102~106 1962
19. Ogasawara, R. : Jour. Japan Forest Soc. **45** ; 319~321 1963
20. Onaka, F. : Bull. Kyoto Univ. Forests **18** ; 55~95 1950

21. Shibaoka, H. and Imaseki, H. : Botanical Magazine Tokyo **70** ; 362～369 1957
22. Slankis, V. : The Physiology of Forest Trees 427～443 1958
23. Stowe, B.B. and Thimann, K.V. : Arch. Biochem. Biophys. **51** ; 499～516 1954
24. Tagawa, T. : Seimeigensho no Kagaku **11** ; 530～534 1961
25. Thimann, K.V. : Ann. Rev. Plant Physiology **14** ; 1～18 1963
26. Varga, M. and Ferenzy, L : Nature **178** ; 1075 1956
27. Went, F.W. and Thimann, K.V. : Phytohormones 1937
28. Wildmann, S.G., Ferri, M.G. and Bonner, J. : Arch. Biochem. Biophys. **13** ; 131～147 1947
29. Wildmann, S.G. and Bonner, J. : American Jour. Botany **34** ; 740～746 1948
30. Zimmermann, W.A. : Zeitschr. Bot **30** ; 209～252 1937

和 文 要 約

アカマツの生長物質および抑制物質に関する研究

小 笠 原 隆 三

アカマツの芽，葉，根に含まれる生長物質および抑制物質を水又はエーテルで抽出し，ペーパー・クロマトグラフィーで分離した後アベナ伸長試験で測定した。

酸性区分の生長促進帯に Ehrlich 試薬で発色する四つの物質が認められた。

このうち1つは IAA 又は IAA と近縁の物質と考えられた。

又若干の抑制物質が酸性，中性区分に認められた。

生長物質は頂部にある芽が最も多く，葉，茎と頂部から離れるにつれ減少し，根において再び増加する傾向がみられた。

生長物質は4月から10月まで認められたが12月から2月までのいわゆる休眠期にはほとんど認められなかつた。

抑制物質は1年を通して存在するが休眠期の方が他の季節よりも多く存在した。

このことから生長物質および抑制物質はアカマツの生長，休眠の調節に密接な関係があるものと考えられた。