Distribution of ^{14}C -sugars from $^{14}\text{CO}_2$ in healthy and leaf-rust-infected poplar leaves

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千葉 修*・高井省三*: 葉さび病菌に対して感受性を異にするポプラの
クローン間の光合成 ^{14}C 糖類生成の差異

Abstract

Three poplar clones, *Populus deltoides angulata* (resistant), *P. deltoides missouriensis* (susceptible), and *P. "Kamabuchi-1"* (variable in susceptibility according to the growing season) were inoculated with uredospores of *Melampsora larici-populina*, incubated for 9 days, and then cuttings from healthy and infected plants were fed with $^{14}\text{CO}_2$ for 20 hours.

In healthy and rusted leaves of these clones, at least six kinds of sugars, sucrose, glucose, fructose, raffinose (?) and two other unidentified sugars, were detected on the autoradio-chromatograms. The specific radioactivity of each sugar generally increased in rusted leaves with a few exceptions, whereas there were remarkable differences in the increasing degree of specific radioactivity among sugar fractions.

Labeling of sucrose, glucose and fructose with ^{14}C from $^{14}\text{CO}_2$ increased by rust infection, and the degree of the increase was conspicuous in *P. deltoides missouriensis*, while it was insignificant in *P. deltoides angulata*. A general increase in both total and specific radioactivity of sucrose may suggest the possibility of stimulated photosynthesis in rusted leaves.

The degree of increase in the specific radioactivity of glucose and fructose were almost equal in rusted leaves of *P. deltoides angulata*, whereas particular increases were observed for glucose in *P. deltoides missouriensis* and for fructose in *P. "Kamabuchi"*, respectively. This fact would suggest that in rusted leaves of the latter two clones the disturbance of carbohydrate metabolism incited by rust infection is still continued, and that there are considerable differences between two clones concerning the pathway of carbohydrate metabolism in infected leaves.

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Introduction

A leaf rust caused by *Melampsora larici-populina* KLEBAHN is one of the commonest and most serious diseases of poplars in Japan, and there are marked differences in the susceptibility to this rust among poplar clones²⁾. In the previous paper²⁾, the senior author suggested that the component of sugars in leaves was a major factor determining the susceptibility to this rust disease; that is, the ratio of the sucrose content to the reducing sugars content (the S/R ratio) in leaves was considerably different among poplar clones, and a condition where leaves of a given clone have a higher S/R ratio in their sugars content would account for its higher susceptibility to this rust.

The relation between the reaction type to rust and the carbohydrate contents of host leaves have been studied by many workers, especially on wheat stem rust^{6,7,9,14)}, bean rust⁵⁾, corn rust¹²⁾, and safflower rust⁴⁾. Many observations suggested a close relationship between carbohy-

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drates level in the host and the development of these obligate parasites. In addition, carbohydrate transformation within host tissues, which appears to be related to respiratory changes due to infection by obligate parasites, have been thought to play a significant role in parasite development. Several workers have confirmed apparently fundamental changes in the pathways of carbohydrate metabolism in host tissues during infection by obligate parasites, using radioisotope tracer techniques^{1,3}.

In the case of the leaf rust of poplars, information about the metabolic changes induced from rust infection is still lacking. The present experiments were made to ascertain the difference in distribution of the ¹⁴C-sugars derived from photosynthetically fixed ¹⁴CO₂ and the effect of rust infection on their distribution among poplar clones which vary in susceptibility to this rust.

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Materials and method

As test materials three clones of poplars were used; *Populus deltoides angulata* (B1-12)--- resistant, *P. deltoides missouriensis* (B1 8)--- susceptible, and *P. "Kamabuchi-1"* (LS-1)--- highly susceptible in autumn, whereas resistant in early summer producing large necrotic spots with a few uredosori. The source of these clones was presented in the previous paper².

Potted stocks of these clones were inoculated by depositing drops of uredospore suspension of *Melampsora larici-populina*, eight to ten per leaf, in the greenhouse on September 16, 1963. On the 9th day after inoculation, the characteristic response for each clone appeared. As shown in plate 1, an apparent sporulation was observed in the clone B1-8, while only small necrotic spots were produced at the inoculation points of the clone B1-12. In the case of the clone LS-1, comparatively wide necrotic lesions over the inoculation points were produced without sporulation. Thus, the response of the clone LS-1 in the present experiment was regarded rather as that of the early summer type of this host-parasite combination.

The rust-infected and healthy shoots bearing 7-8 leaves from the top, were cut off from the stocks and each was put in a flask containing water. Then, they were placed in an apparatus of a glass bell jar for photosynthesis, where the atmosphere was enriched with ¹⁴CO₂ generated from Ba¹⁴CO₃ (250 μ c) with lactic acid for 20 hours in light at about 20°C. After incubation, leaves were cut into pieces and extracted with hot 80 % ethanol 3 times. After evaporating ethanol, the water soluble portion was shaken with acetone and then with ether, added upon it, in an attempt to remove chlorophyll. The ether layer was carefully washed with water to recover the sugars into water. The water soluble fraction was passed through Amberlite IR 120 and IRA 410 columns. The effluent from both columns, consisting of a sugar fraction, was concentrated to a definite volume and was submitted to one-dimensional ascending paper chromatography on Toyo-roshi No. 51 A filter paper, with butanol-acetic acid-water (6:1:2). Radioactive spots were located by autoradiography using Fuji X-ray film. Every sugar spot on the chromatogram was eluted with hot water exhaustively. The radioactivity of dried elute was counted by an end window G.M. counter.

Results and discussion

a) Detection of sugars on chromatograms

At least six bands of sugars were detected on the autoradiogram in every case (Plate 1). The sugars on four of these six bands were identified as sucrose, glucose, fructose, and raffinose(?), respectively. Two other bands, one of which was retained at the starting point, were unidentified.

Although there is noticeable discrepancy between their data, Jain & Pelletier (1958) and Wang (1960) detected sedoheptulose in leaves of the wheat variety Little Club, susceptible to the stem rust. The former authors reported that accumulation of ^{14}C in sedoheptulose was greater in rusted leaves at the seven-day stage after inoculation than in healthy ones, and such situation was suggested as being the most striking metabolic alternation caused by infection. On the other hand, Wang's experiment gave a result, in which the accumulation was rather greater in healthy leaves than in rusted ones ten days or more after inoculation. Besides, according to Daly (1962), trehalose was one major component synthesized in rust-infected safflower hypocotyls, and Trip *et al.* (1963) declared that mannitol was a metabolically active product from $^{14}\text{CO}_2$ on several tree species, such as lilac and ash trees.

In this study, however, the authors were unable to confirm these sugars and sugar alcohol both in healthy and rusted poplar leaves. It would be an interesting subject of future research to determine whether the present results were brought about by the difference in tested materials or by the condition under which this experiment was conducted.

b) Radioactivity of the sugars

Specific activity of six kinds of sugar fractions are shown in table 1.

Table 1. Distribution of specific radioactivity of sugar fractions in healthy and rust-infected poplar leaves.

Clone	Leaves	Specific radioactivity of sugar fractions (cpm/mg)					
		I ^a	II	III	IV	V	VI
<i>deltoides</i> (B1-12)	Diseased (D)	898	1680	415	10060	1031	908
	Healthy (H)	130	454	480	3996	899	683
	D/H	6.9	3.7	0.9	2.5	1.2	1.3
<i>deltoides</i> (B1-8)	Diseased (D)	744	2680	175	7608	938	406
	Healthy (H)	286	513	129	3390	366	330
	D/H	2.6	5.2	1.4	2.2	2.6	1.2
"Kamabuchi-1" (LS-1)	Diseased (D)	168	1016	178	9716	1405	1033
	Healthy (H)	320	843	137	4542	847	360
	D/H	0.5	1.2	1.3	2.1	1.7	2.9

^a I : unidentified A, II : raffinose (?), III : unidentified B, IV : sucrose, V : glucose, VI : fructose.

Among these sugars, both specific and total radioactivity was highest in sucrose in all cases of healthy plants as well as rusted ones. This fact would be reasonable, because sucrose is a major direct product of photosynthesis. With respect to variation in specific radioactivity of each sugar induced from rust infection, a general increase was observed, excepting a slight reduction in the unidentified B of the clone B1-12 and the unidentified A of the clone LS-1. The general increase in specific radioactivity of sugars would be explained by the well-known fact that

infection causes an enhancement of metabolic activity, above all of respiration.

On the other hand, there were conspicuous differences in the increasing degree of radioactivity among sugar fractions. A particularly great increase of specific radioactivity was found on the unidentified A of the clone B1-12 and on raffinose (?) of the clone B1-8, whereas no significant difference in the degree of increase of specific radioactivity of sucrose was found among all clones.

Table 2 shows the distribution of total radioactivity of three kinds of sugars; sucrose, glucose and fructose, in healthy and rusted leaves.

Table 2. Distribution of total radioactivity of sucrose, glucose, and fructose in healthy and rust-infected poplar leaves (per g fresh wt.).

Clone		Healthy leaves		Diseased leaves		D/H in cpm
		Amount mg	Total activity 10 ³ cpm	Amount mg	Total activity 10 ³ cpm	
<i>deltooides</i> (B1-12)	S ^a	38.8	155	24.7	249	1.6
	G	23.3	21	21.0	22	1.0
	F	28.0	19	18.5	17	0.9
	Tot.	90.1	195	64.2	288	1.5
<i>deltooides</i> (B1-8)	S	46.6	158	50.0	380	2.4
	G	21.7	8	20.0	19	2.4
	F	15.5	5	32.4	13	2.6
	Tot.	83.8	171	102.4	412	2.4
'Kamabuchi-1' (LS-1)	S	39.1	177	27.5	266	1.5
	G	11.7	10	13.2	19	1.9
	F	13.7	5	7.7	8	1.6
	Tot.	64.5	192	48.4	293	1.5

^a--- S : sucrose, G : glucose, F : fructose.

In rusted leaves of all tested clones, incorporation of ¹⁴C from ¹⁴CO₂ into these sugars increased and the degree of increase was conspicuous in the susceptible clone, B1-8, whereas in the resistant B1-12 a slight increase was observed only on sucrose.

A general increase in total and specific radioactivity of sucrose may suggest the possibility of stimulation of photosynthesis in rusted leaves. On the contrary, in the case of stem-rusted wheat a decrease in radioactivity of sucrose was reported by Jain & Pelletier (1958) and Wang (1960). Besides, Shaw (1954) and Livne (1962) reported that infection caused a depression of photosynthesis in wheat and bean, although Livne added that the stimulated photosynthesis in the rust-free leaves adjacent to the diseased leaf was assumed in the rusted bean plant. Consequently, it may be suggested that the possible stimulation of photosynthesis would be due to the method of this experiment, in which inoculation was made for relatively small parts on the whole leaf (Plate 1).

It is well known that photosynthetically fixed ¹⁴CO₂ labels sugars via 3-phosphoryl-D-glyceric acid, fructose-1,6-diphosphate, fructose-6-phosphate, and glucose-6-phosphate, in the 3, 4 positions of hexoses, and the specific radioactivities of glucose and fructose are almost equivalent. In the present experiment, this relationship between glucose and fructose was almost normally maintained in the resistant clone B1-12. However, such a relation between glucose and fructose in healthy leaves was obviously altered in the rusted leaves of the other clones, B1-8 and LS-1 (Table 1). In the clone B1-8, a particular increase in specific radioactivity

of glucose was observed, while a great increase in that of fructose was found in the clone LS-1. It seems that the effect of infection on the carbohydrate metabolism of the two clones resulted in an increase of both total and specific radioactivity of these sugars, but it coincidentally brought about the disturbance of carbohydrate metabolism.

The variance in the degree of increase in total radioactivity and also in the above-mentioned relation between glucose and fructose among the clones may give a suggestion to an aspect of the host metabolic change in rusted leaves. The increase in total radioactivity as well as specific activity of the three kinds of sugars may suggest that the enhanced metabolic activity has been kept during the incubation period in the susceptible clone, B1-8. On the contrary, in the case of resistant clone B1-12, the metabolic activity, supposedly stimulated at the earlier stage of infection, has returned to the normal condition at the tested period. This assumption would be supported by the unpublished results of the other experiment, in which the uptake of ^{32}P in infected leaves was still active six days after inoculation in the clone B1-8, while that in the clone B1-12 became insignificant at the time.

It is well known that these sugars are readily utilized as a substrate of respiration. Consequently, increase in both total and specific radioactivities of these sugars seems to indicate that a demand for these sugars as a substrate for host and parasite respiration became greater. Shaw (1963), in his review on the physiology and host-parasite relations of the rusts, has presented several examples of the respiratory change accompanied by rust infection. A shift from the Emden-Meyerhof-Parnas pathway in favour of the pentose phosphate pathway has been demonstrated in several host-parasite combinations. Daly *et al.* (1962) reported that the shift abruptly occurred when the sporulation was started. Accordingly, the results of the present experiment on the radioactivity of glucose and fructose would suggest that there are considerable differences between the clones B1-8 and LS-1 concerning the shift in the pathway of carbohydrate metabolism and also the subsequent physiological changes, including defence reactions such as browning reaction, utilizing these sugars as substrates, although we have not yet sufficient data for evaluating this possibility on rusted poplar leaves. Further studies therefore would be needed to make clear the time of onset and extent of the increase in respiration, and also the shift of the respiratory pattern on diseased leaves which have more heavy and earlier stage of infection.

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Plate I

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Explanation of plate

Plate 1.

A—C : Variation in reaction of leaves of three poplar clones inoculated by *Melampsora larici-populina*. (8 days after inoculation)

A : *Populus deltoides angulata*, B : *P. deltoides missouriensis*,

C : *P. "Kamabuchi-1"*.

D—F : Radioautographs of chromatograms of the ^{14}C -sugars from $^{14}\text{CO}_2$ in the healthy (left) and rust-inoculated (right) leaves of three poplar clones. I, II, III, IV, V, and VI represent unidentified A, raffinose (?), unidentified B, sucrose, glucose, and fructose, respectively.

D : *P. deltoides angulata*, E : *P. deltoides missouriensis*, F : *P. "Kamabuchi-1"*.

和文摘要

葉さび病菌に対して感受性を異にするポプラのクローン間の 光合成 ^{14}C 糖類生成の差異

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葉さび病菌 (*Melampsora larici-populina*) に対して感受性を異にするポプラの3クローンを使用して、接種9日後の罹病葉および無接種葉に $^{14}\text{CO}_2$ を与え、生成される ^{14}C 糖類の差異を調べた。いずれの供試葉からも、オートラジオ・クロマトグラム上で、ブドウ糖、果糖、蔗糖、およびラフィノース(?)を含む少なくとも6種の糖の存在を認めた。これら6種の糖の比放射能 (cpm/mg) は、一般に罹病葉で増加することを認めたが、その増加の割合は、クローンまたは糖の種類によつてかなり異なる。

蔗糖・ブドウ糖・果糖への ^{14}C のとりこみは、罹病によつて、感受性クローンでは顕著に増加し、炭水化物代謝活性の高まりがなお継続されていることを示したが、抵抗性クローンでは変化は認めなかつた。

蔗糖は他の糖とくらべて著しく高い放射能を示し、また、いずれのクローンでも罹病による増加を認めたが、このことは罹病葉における光合成能の促進を暗示する。

一方、ブドウ糖および果糖についてみると、罹病による放射能の変化には、クローン間で著しい差異が認められた。