Much more phytoalexin was produced by the attached than by the detached leaf. A high spore concentration increased both the rate and amount of phytoalexin over a low concentration. A predisposing temperature of 70° F produced more inhibitory substances than did 80-90° F. Diffusates from seedlings inoculated at the fifth and sixth leaf stage inhibited spore germination more than seedlings inoculated at later stages. Production of phytoalexin increased up to the third, fourth or occasionally the fifth day after inoculation. After this time, the inhibitory effect gradually disappeared.

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1. A new anthocyanidin in maize: luteolinidin.

The gene sm (salmon silk) in the presence of red pericarp (P^{rr}, etc.) results in silks which are salmon in color. The P locus determines pericarp and cob color. Of particular interest is the P^{rr} allele which gives red pericarp and cob color (Emerson et al., 1935). This study was directed to the identification of pigments which cause salmon silk and red pericarp color in the genotype P^{rr} sm/P^{rr} sm.

A genetic marker stock, P^{rr} Y Pl sm py/P^{rr} Y Pl sm +, which has salmon silks, red pericarp, and red cob was used for this study. Silks from this stock about 5 days after emergence were removed from the ear and ground in acidified 80% methanol. Pericarp from mature kernels of the same stock was obtained by soaking kernels for 4 hours and hand-peeling the pericarp. Similarly, pericarp was ground in acidified 80% methanol. The pericarp and silk samples were allowed to stand for 24 hours, filtered, and reduced at 40° C with a rotary evaporator. Separation and identification of anthocyanins was accomplished by the usual techniques, paper chromatography, acid hydrolysis, and light and UV

spectrophotometry (Harborne, 1967). Four solvent systems were employed for paper chromatography with No. 1 Whatman paper. The solvents were: BAW, n-butanol-acetic acid-water (4:1:5, top layer); 1% HCl, water-conc. HCl (97:3); HOAc-HCl, acetic acid-conc. HCl-water (15:3:82); and Forestal, acetic acid-conc. HCl-water (30:3:10). Spectral determinations first were made in methanol containing .01% conc. HCl and then after the addition of 6 drops of a 5% $AlCl_3$ solution.

Acid hydrolysis of salmon silk extracts yielded one anthocyanidin which was orange in color. R_f values and spectral maxima are presented in Table 1. For comparison, R_f values and spectral maxima for luteolinidin are cited (Harborne, 1967). R_f values for the salmon silk anthocyanidin and luteolinidin are similar in Forestal and BAW solvents. Spectral maxima for the two pigments were also similar in MeOH-HCl as were the bathochromic shifts due to the $AlCl_3$ reagent. Based upon these similarities, the unknown anthocyanidin derived from salmon silks is identified as luteolinidin.

Table 1. Colors, R_f Values and Spectral Maxima of Salmon Silk Pigments

Anthocyanidin	Visible color	R_f values (x 100) in				MeOH-HCl	$AlCl_3$
		Forestal	BAW	1% HCl	HOAC-HCl	λmax(nm)	$\Delta\lambda$(nm)
Unknown Anthocyanidin (hydrolysate)	orange	63	61	4	17	499	50
Luteolinidin*	orange	61	56	--	--	493	52
Anthocyanin							
No. 1	orange	--	27	17	39	--	--
No. 2	orange	--	57	33	42	--	--

*Values as cited by Harborne (1967).

Two anthocyanins based on luteolinidin have been isolated from salmon silks. Color and R_f values are reported in Table 1. No. 1 has

R_f values like luteolinidin 5-glucoside (Harborne, 1967) and is the most abundant. The identity of No. 2 is unknown.

The same pigments were found in the red pericarp ($\underline{P}^{rr}$) as in salmon silks. Results given in Table 1 apply equally well for red pericarp and are not repeated. Red pericarp color is, consequently, due to two anthocyanins based on luteolinidin.

Red silk color is common in corn and is easily distinguished from salmon. This red pigment was isolated from the silks of the inbred line NC 236 by the methods previously described. The anthocyanidin was prepared by acid hydrolysis and purified by paper chromatography. Two anthocyanidins, cyanidin and pelargonidin, were identified by co-chromatography with authenticated anthocyanidins in two solvent systems. These two anthocyanidins have previously been reported to be responsible for plant and aleurone color in corn.

It, thus, appears that two distinct pigment systems exist for the determination of silk color. The first is red silk color, which is based upon anthocyanins of cyanidin and perlargonidin. Red silks are determined by certain $\underline{R}$ alleles ($\underline{R}^{rr}$, $\underline{r}^{rr}$, etc.) when $\underline{A}_1$ is present (Emerson et al., 1935). The second is salmon silk color, which is based upon anthocyanins of luteolinidin. Salmon silk is determined by the $\underline{sm}$ allele when certain $\underline{P}$ alleles ($\underline{P}^{rr}$, etc.) are present.

A structural comparison of luteolinidin versus cyanidin and pelargonidin is revealing. Luteolinidin is deoxy for the 3 position while cyanidin and pelargonidin are hydroxylated. This suggests that 3-deoxy compounds, flavonones or flavones, would be likely precursors of luteolinidin. On the other hand, 3-hydroxyl compounds, dihydroflavonol or flavonol, would be likely precursors of cyanidin and perlargonidin. The need for different precursors could account for the two distinct genetic systems controlling salmon and red silk color.

References:

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