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Identification and Localization of Vascular Occlusions in Cut Roses[1]

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Abstract. **Microscopic examination of longitudinal tangential sections of stems of rose (*Rosa hybrida* L. cv. Red American Beauty) revealed vascular occlusions due to microbial growth and gum deposition. Microbial occlusions reacted positively with the protein stain mercuric bromphenol blue and were restricted to the basal 2.5 cm of the stem. Gum deposition was identified by a positive reaction with periodic acid-Schiff's reagent and gas liquid chromatographic analysis of monomer content of acid-hydrolyzed occluding material. Location of gum deposition was dependent on the depth of the holding solution on the stem, always occurring above the solution level. Quantitative and qualitative comparison of sugar and uronide monomer content between areas of no gum deposition and areas of high gum deposition showed no differences, suggesting that gum formation was due to redeposition rather than net synthesis of gum constituents.**

Decreased water uptake due to vascular occlusion has been associated with the decreased vaselife of cut roses (1, 2, 5, 15). Various factors which contribute to vascular occlusion and increased solution flow resistance have been observed. Burdett (2) found that stem sterilization or 8-hydroxyquinoline solutions controlled microbial growth and decreased solution flow resistance. Gilman and Steponkus (7) showed that 8-hydroxyquinoline sulfate (8-HQS) maintained the xylem conductivity of isolated stem sections at a high level concomittantly increasing vaselife of cut roses. Examination of stem sections by scanning electron microscopy has revealed the occurrence of bacterial and fungal occlusions in roses held in 2% sucrose (18).

Vascular occlusions not attributable to microbial contamination have also been observed. Marousky (15) reported that roses held in sterile solutions of 8-hydroxyquinoline citrate had less vascular blockage and greater solution uptake than roses held in sterile water implying vascular occlusions other than microbial were involved. Burdett (2) found occlusions which were characterized as pectinaceous in nature and hypothesized that these occlusions were due to the secretion of enzymes by microorganisms which converted cell wall materials to the substances which occluded the vessels. Parups and Molnar (17) extended the cytohistochemical investigation on "pectinaceous" occlusions and found the material to contain carbohydrates, pectin, protein and lipid-like compounds. An active process of stem tissue which could account for occlusion of a carbohydrate nature is gum formation. Gums have been observed in woody stems occurring as physiological responses to infection, wounding, and heartwood formation (6). Rasmussen and Carpenter (18) have recently observed occlusion of vessel elements in addition to microorganism growth which they interpreted as due to the breakdown of the secondary tissue of the xylem.

In order to fully characterize "carbohydrate" vascular occlusion one must examine both the location of occurrence and chemical identity and make comparisons to known cellular carbohydrate distribution. Results will be presented which locate the area of occlusion in a cross-sectional and longitudinal profile of the rose stem and describe the chemical composition by staining reaction and chromatographic separation of its monomer subunits.

Materials and Methods

Experimental materials used were 'Red American Beauty' roses grown under standard cultural practices in the Cornell Univ. floriculture greenhouses. Roses were cut to 40 cm length and placed in solution immediately after harvest. Flowers were held under a 12-hr photoperiod (fluorescent light 700 lux) at 27°C, and approx 65% relative humidity. Stem sections for the observations were taken following the appearance of "bent neck."

Cytohistochemical observations. Stem sections 2.5 cm in length representing the entire longitudinal profile of the stem were fixed in formalin-acetic acid-alcohol (FAA) (10), dehydrated through a tertiary butyl alcohol (TBA) series (8), and embedded in Tissuemat. Longitudinal, tangential sections were cut to 20 μ thickness on a rotary microtome, mounted on glass slides and stained with periodic acid-Schiff's reagent according to the schedule of Jensen (10).

Fresh longitudinal, tangential sections were cut on a sliding microtome and stained with a commercial preparation of safranin-Delafield's hematoxylin, PAS, or mercuric bromphenol blue (16). Fresh transverse sections were hand-cut and stained as above or observed unstained by bright field light microscopy.

Solution effects. Roses were placed in the modified Cornell solution (200 mg/liter 8-HQS + 2% sucrose) at a constant solution depth of 7.6 or 12.7 cm. Transverse sections were taken at 2.5 cm intervals in an acropetal direction and microscopically observed unstained. Amount of vascular occlusion was expressed as the percentage of vessel members occluded relative to the number of vessels exposed. This technique was also applied in evaluating the effects of the modified Cornell solution and potassium azide (KN_3) on location and magnitude of gum occlusion. KN_3 was evaluated at a concentration of 100 mg/liter. Solution depth was maintained at 10 cm.

Identification of occluding material by gas chromatography. Strings of occluding material occurring above the solution level in the modified Cornell solution were manually extracted from vessels of longitudinal stem sections using 30× magnification and a hand-held microneedle. The material was subjected to hydrolysis in hydrochloric acid (HCl) or trifluoroacetic acid (TFA). HCl hydrolysis was achieved by heating the sample in 1 N HCl at 90°C for 8 hr. TFA hydrolysis was conducted by placing the occlusions in 0.4 N TFA in a sealed container and heating in a steam autoclave at 121° and (1 kg/sq. cm) 15 psi for 1 hr. Following drying *in vacuo* and lyophilization, silated derivatives of the hydrolysates were prepared essentially according to Kaltaler and Steponkus (13). Derivatives of hydrolyzed occluding material and standards of rhamnose, arabinose, xylose, mannose, galactose, glucose, fructose, sucrose, galacturonic acid, glucuronic acid and benzoin (internal standard) were chromatographed on a Varian model 1840 gas chromatograph. Chromatographic columns were 3.8 m × 3 mm (12.5 ft

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by 1/8 inch) stainless steel packed with Varaport #30 (100/120 mesh) solid phase and 2% QF-1 liquid phase. The hydrogen flame ionization detector and injector temp were maintained at 225°C. The column oven was programmed in 20 increments, from an initial temp of 145°, 2 increments at 8°/min, followed by 1 min steps of 6°/min. Nitrogen (20 cc/min) was used as the carrier gas. Oxygen and hydrogen flow rates were 300 and 30 cc/min, respectively. Electrometer response was graphically recorded and integrated by a Varian model 840 integrator.

Quantitative comparison of xylem hydrolysates. Quantitative comparison of xylem hydrolysate monomer content from areas of much occlusion and no occlusion was conducted. In the initial experiments stem sections above and below the holding solution level represented areas of high or no occlusion, respectively. The bark was stripped from stem sections, sections were lyophilized, then ground in a Wiley mill (#40 mesh screen). Extraction with 0.5% ammonium oxalate was carried out at 90°C for 8 hr (19), and the uronic acid content of the resultant hydrolysates was quantitated colorimetrically according to the carbazole technique described by Dische (4). HCl hydrolysis, derivative formation and chromatography of samples from the two tissue areas were carried out as before. Subsequent experiments utilized sections from above the solution level of stems held in the modified Cornell solution (MC) or MC + 100 mg/liter KN_3 as tissues with or without gum occlusion, respectively. TFA hydrolysis, derivative formation, and chromatographic separation were achieved as outlined for identification of occluding material.

Results and Discussion

Microscopic examination of serial longitudinal sections of rose stems presented a profile of the xylem and allowed observation of any sources of occlusion. Two types of vascular occlusion were found in stems of roses held in distilled water. These occlusions were distinguishable by staining reaction, general appearance, and location of occurrence in the stem profile.

Basal portions of the stem were occluded by a dense, granular material which gave positive staining response to safranin and mercuric bromphenol blue. These staining responses and the texture of the occluding mass implied bacterial contamination to be responsible for the basal occlusion (Fig. 1) which was restricted to the first 2.5 cm of the stem.

A second source of occlusion occurred above the solution level on the stem. This material was homogeneous in texture and failed to react with mercuric bromphenol blue. A strongly positive reaction with periodic acid-Schiff's stain (PAS) indicated this material to be carbohydrate in nature. Occlusions of this type could be observed both as discrete globules occurring along vessel walls adjacent to ray parenchyma cells (Fig. 2) and as strings completely filling the vessels (Fig. 3). Intermediates between the two could also be observed indicating the small globules probably coalesced to form the occluding strings. This interpretation was based on a developmental hypothesis rather than an actual time-course study, however. A transverse section of rose xylem observed unstained revealed the magnitude of occurrence of the carbohydrate containing occlusions (Fig. 4).

The relationship between the level of the holding solution on the stem and the locus of occurrence of the "carbohydrate" occlusion was demonstrated by comparing serial transverse sections from stems held at two constant solution levels. Lowering the solution level on the stem by 5 cm displaced the locus of first occurrence of the occlusions by the same distance (Fig. 5).

Occurrence of two types of vascular occlusion in cut flowers has been documented. Aarts (1) observed slimy accumulations

Fig. 1. Longitudinal, tangential section of basal portion of rose stem stained with mercuric bromphenol blue. Occlusion of the vessel element in the center of the micrograph is evident (arrow). Horizontal bar represents 40 μ.

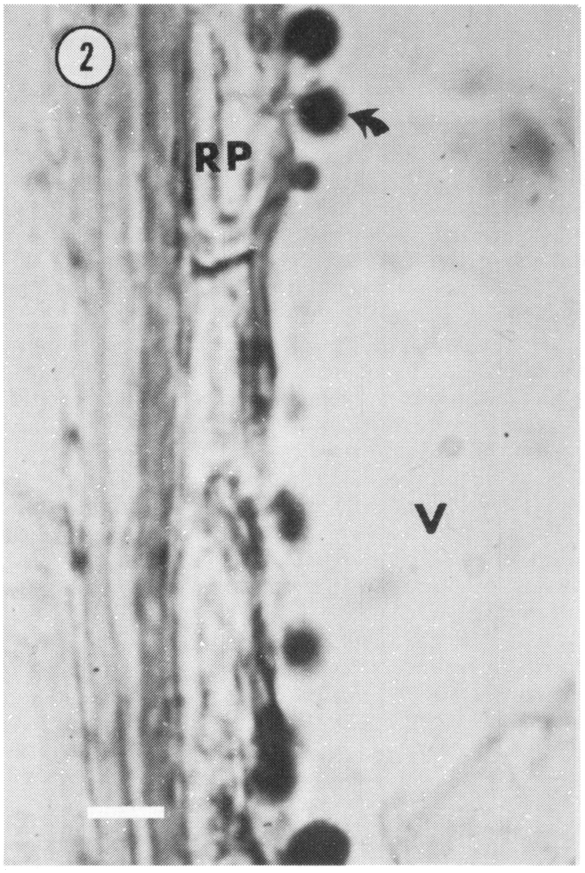

Fig. 2. Longitudinal, tangential section of rose xylem; fixed, dehydrated, and stained with periodic acid-Schiff's reagent. Densely stained globules (arrow) are seen in the lumen of the vessel element (V) proximal to ray parenchyma cells (RP). Horizontal bar represents 6.25 μ.

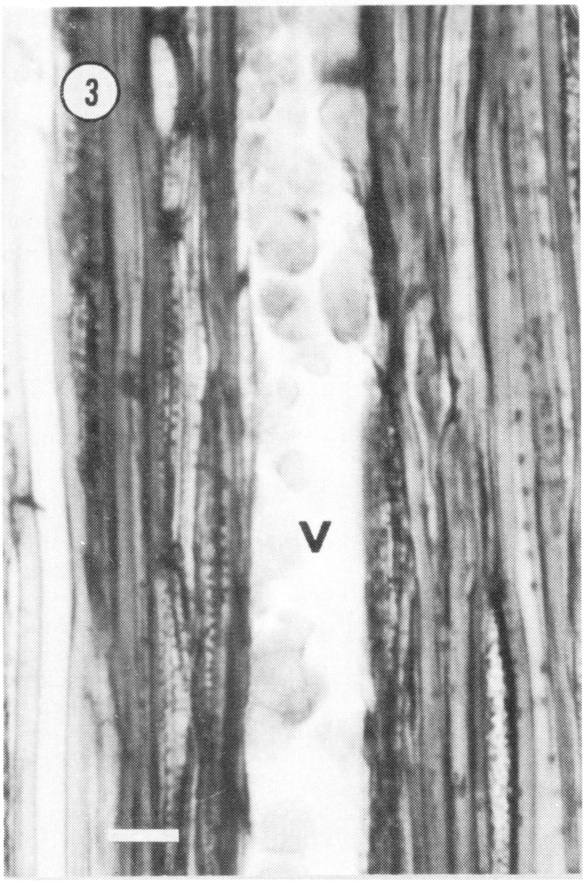

Fig. 3. Longitudinal, tangential section of rose xylem taken above the solution level; stained with periodic acid-Schiff's reagent. The lumen of the vessel element (V) is completely occluded at one locus (upper center of micrograph). Horizontal bar represents 40μ.

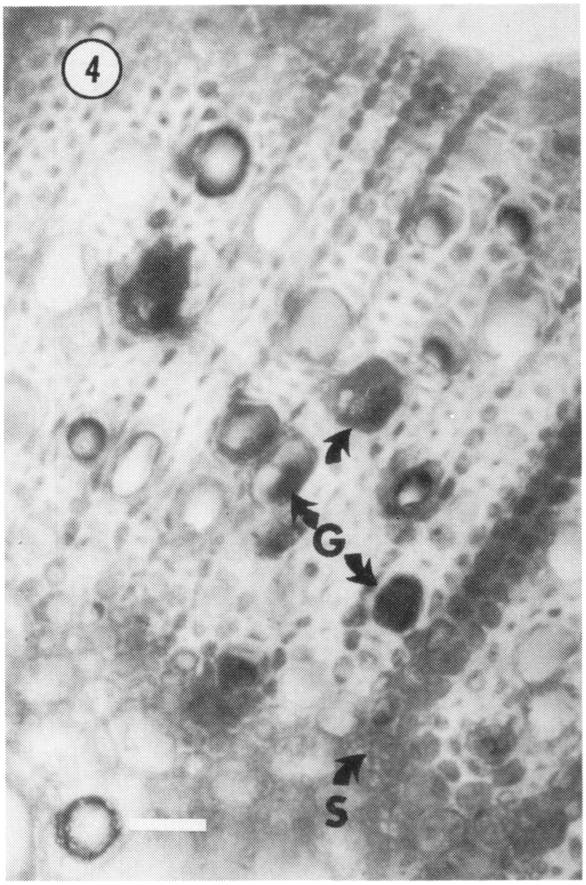

Fig. 4. Transverse section of rose xylem above the solution level observed unstained. A distinction can be made between vessels containing starch (S) introduced as an antifact during sectioning, and those containing gums (G). Horizontal bar represents 100μ.

of bacteria and breakdown products occurring on the cut surface and occluding the vessels of *Alnus, Chrysanthemum* and *Dianthus.* Occurrence of these microscopically observable occlusions was correlated with a simultaneous decrease in the potential conductivity of stem sections. Burdett (2) found sterilization of stems and holding solutions to reduce vessel plugging from bacteria and decrease flow resistance in stem sections of *Rosa.* Addition of the bacteriostatic agent, 8-hydroxyquinoline, also decreased vessel plugging.

Vascular occlusion not due to microbial contamination has also been described. Aarts (1) observed a "gummy" substance occurring in the stems of *Alnus* which occurred above the solution level on the stem. Cytohistochemical studies by Burdett (2) and Parups and Molnar (17) indicated similar occlusions in rose stems which were characterized as being "pectinaceous" in nature. On the basis of scanning electron microscopy, this type of occlusion has also been referred to as breakdown products of secondary wall material (18).

Durkin and Kuc (5) hypothesized that vascular occlusion began at the basal end of the cut flower and progressed acropetally with time. The data presented here and in other literature is consistent with two types of vascular occlusion from two unrelated sources, rather than one type extending through the stem profile. Bacterial occlusion is restricted to the basal portions of the stem. A second type occurs above the solution level on the stem, and is characterized as carbohydrate in nature.

The carbohydrate occlusion morphologically resembles the gums formed in vessels of heartwoods described by Chattaway (3). Air filled vessels bordering ray parenchyma cells are believed to be the stimulus for altered ray cell metabolism resulting in gum formation. If gum formation in vessels is an oxidative process, presence of gum occlusion only above the solution level as was found here in stems of *Rosa* and by Aarts in *Alnus* (1) would be expected.

Limitations due to the lack of specificity of cytohistochemical techniques have prevented more precise characterization of the "carbohydrate" occlusions and hindered further research into the nature of occurrence of this type of vascular occlusion. Hence, the technique of acid hydrolysis of occluding material and comparison of component monomers to the known

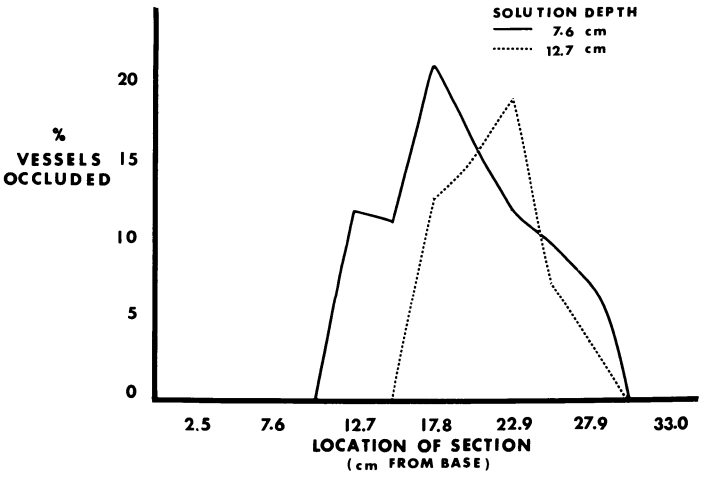

Fig. 5. Occurrence of PAS positive occlusions as a function of depth of holding solution (held in modified Cornell solution).

Table 1. Identification of the components of HCl and TFA hydrolysates of carbohydrate occlusions and xylem tissue.

Standard	Standard elution range[z]	HCl hydrolysis Xylem	HCl hydrolysis Occlusions	TFA hydrolysis Xylem	TFA hydrolysis Occlusions
rhamnose					
α	.46–.49	.43		.48	.46
β	.54–.57			.56	
arabinose					
α	.51–.52	.46	.53	.50	.52
β	.53–.55	.48		.53	.55
xylose					
α	.60–.61	.58		.60	.59
β	.64–.65	.62		.63	.63
mannose					
α	.67–.68	.66	.66	.67	.66
β	.81–.82	.80		.84	.80
galactose					
α	.75–.76			.74	.74
β	.81–.82	.80		.80	.80
fructose					
α	.67–.68				
β	.71–.72				
glucose					
α	.76–.77	.75	.75	.76	.76
β	.89–.90	.88	.87	.88	.88
galacturonic acid					
α	1.04	1.07	1.08		
β	1.06	1.07	1.08		
glucuronic acid					
α	.98				
β	1.04				

[z]Elution range relative to internal standard, benzoin.

monomer content of classes of cellular carbohydrates was used to identify the occluding material specifically. Gas liquid chromatographic analysis of acid hydrolyzed occluding material yielded the monomers presented in Table 1. HCl hydrolysis was apparently incomplete since strings of occluding material could be observed in the acid solution following heating. A closer estimate of the monomer content was obtained by TFA hydrolysis. However, galacturonic acid was not detected in TFA hydrolyzed samples due to breakdown under the severe hydrolysis conditions (12). Through the hydrolysis technique monomers of rhamnose, arabinose, xylose, mannose, galactose, glucose, and galacturonic acid were detected. Estimates of the molar ratios could not be made because of the small quantities of free occlusions obtainable and low yield of acid hydrolysis under these conditions.

Analysis of HCl and TFA extracts of xylem tissue revealed the same component monomers indicating the qualitative similarity between the isolated occluding material and the rose xylem (Table 1). Rhamnose, xylose, and galactose were detected in HCl hydrolysates of rose xylem, but were not found in HCl hydrolysates of occluding material apparently due to the occurrence of only trace amounts. Their occurrence in the occluding material was indicated by TFA hydrolysis. Glucuronic acid and fructose were not detected in the xylem or occluding material hydrolysates.

On the basis of monomer content coupled with staining response and physical characteristics, the occluding material can be classified into the category of plant gums. Gums differ greatly in relative composition and properties, but are generally composed of various pentoses, hexoses, and uronides (20). Gums are formed in vascular tissue during the process of heartwood formation or as physiological responses to wounding or infection (3, 21). Since examination of a stem profile from roses senescing on the plant revealed no occlusion, and since occlusion can occur in stems held in sterile solution (15), gum formation in the rose stem is likely a physiological response to

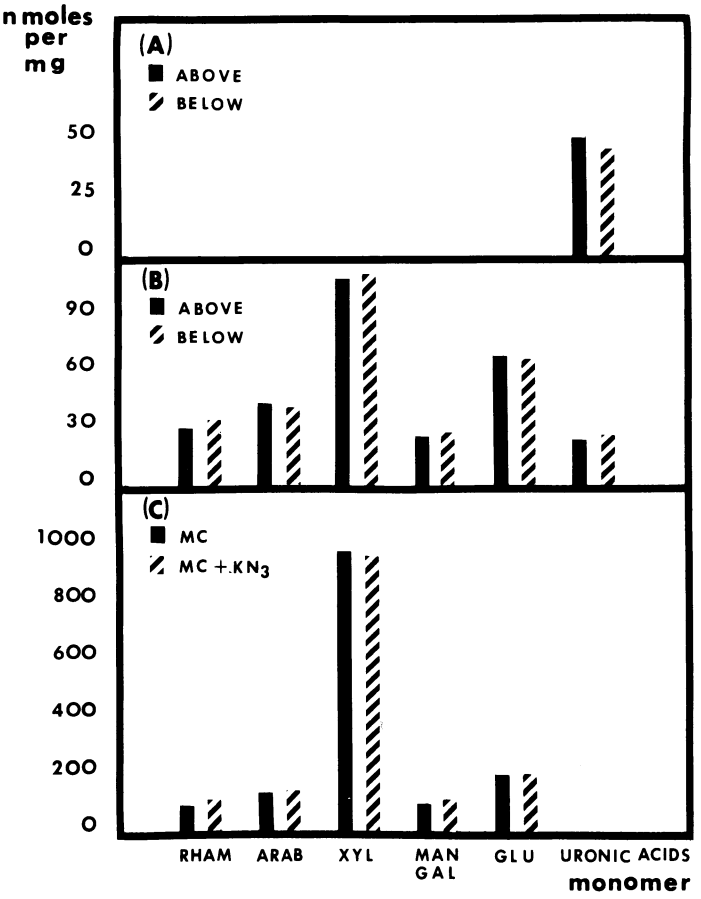

Fig. 6. Quantitative comparison of sugar and uronide content of rose xylem with much (above and MC) or no gum occlusion (below and MC + KN_3). (A) Carbazole analysis of 0.5% ammonium oxalate extracts. (B) GLC analysis of 1 N HCl extracts. (C) GLC analysis of 0.4 N TFA extracts.

wounding. Gum exudation from wounded rose stems has been observed (9).

Gum deposition in rose xylem could occur due to net synthesis of component monomers or redeposition of degraded cellular pectic polysaccharides. Net synthesis of component monomers induced during gum formation would entail areas of much gum occlusion having quantitatively higher monomer content per unit dry weight. Redeposition of degraded cellular pectic fractions into gums formed in the vessels would be reflected by quantitatively similar monomer content in occluded and nonoccluded tissue since a lateral relocation of components would be predicted. Since gum formation has been shown to be restricted to stem tissue above the solution level on the stem, quantitative comparison of gum components in tissues above or below the solution level should reflect which mechanism was involved.

Gums and related pectinaceous polysaccharides are rich in uronides (20) which can be extracted by ammonium oxalate and quantitated colorimetrically by the carbazole technique. Comparison of xylem extracts of tissue above and below the solution level revealed no quantitative differences in uronide content implying redeposition of uronides occurred (Fig. 6A). Further evidence favoring the redeposition hypothesis was obtained by comparing HCl extracts of xylem tissue above and below the solution level (Fig. 6B). Again no differences were detected in amount of gum component monomers when the sugar and uronic acid components were individually characterized.

The preceding argument assumed that xylem tissues above and below the solution level contained similar amounts of gum

component monomers and that hydrolysis proceeded at similar rates. These assumptions may not be valid since the tissues compared were of different morphological and physiological states of maturity. More accurate estimates of the redistribution of pectic substances occurring during gum formation can be made comparing tissues at the same level in the stem. Stem sections above the solution level from roses held in the modified Cornell solution (MC) contain much microscopically observable gum occlusion. Addition of 100 mg/liter KN_3 to the modified Cornell solution eliminates gum formation in 'Red American Beauty' rose stems (14). These data are in agreement with Aarts' (1) finding that NaN_3 depressed "gumming" in stems of *Alnus* developing in holding solutions. Tissues at the same level in the stem from roses held in MC or MC + KN_3 represent tissues of the same state of maturity with much (MC) or no gum occlusion (MC + KN_3). Again no quantitative differences in gum component sugars were detected (Fig. 6C). Estimation of galacturonic acid content of the tissues could not be obtained by TFA hydrolysis because of the breakdown loss discussed previously.

Lack of qualitative and quantitative differences in gum component monomers in tissue exhibiting much or no gum occlusion supports the hypothesis that gum occlusion in rose stems is the result of redeposition of existing gum components rather than increased synthesis of new or existing components. This idea is consistent with the observation of Gruss (as cited by Higgins (9)) who speculated the increased lumen size of wood parenchyma cells in the area of gum formation to be due to the relocation of cellular and middle lamellar pectic and hemicellulosic fractions into gums formed in the xylem vessels.

Occurrence of two distinct types of vascular occlusion is well documented in the literature. Agreement exists that one type is due to the growth of microorganisms (1, 2, 15, 18). The second type has been variously described as a gummy substance (1), pectinaceous or carbohydrate in nature (2, 17), or as breakdown products of secondary wall material (18). Furthermore, the carbohydrate type occlusion has been found to occur above the solution level on the stem (1, 2, 17). In this paper anatomical, cytohistochemical, and biochemical evidence has been presented which would indicate the carbohydrate type occlusion to belong to the category of plant gums. Since this characterization is compatible with the results of previous research (1, 2, 17) we propose that this terminology be adopted when describing occlusion of this type in an attempt to unify the known aspects of vascular occlusion of rose stems.

The demonstration and identification of two types of vascular occlusion due to unrelated sources is fundamental to improving the effectiveness of floral preservative solutions. While previous research (1, 2, 15) has recognized the need for controlling vascular occlusion resulting from microorganisms, it is clear from this and earlier research (2, 17) that such measures do little to prevent occlusion attributable to gum formation. While KN_3 can effectively control both gum and microbial occlusion, its phytotoxic effects negate its practical use. Demonstration that a redeposition of gum components occurs during formation of vascular occlusion should direct attention to formulations which will inhibit this redeposition or prevent its accumulation specifically.

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