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Exogenous sugars overcome incompatibility in hazelnut (*Corylus avellana* L.)*

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Summary. Petreatment of hazelnut (*Corylus avellana* L.) stigmas with 1 M or 2 M sugar solutions before pollination with incompatible pollen, or pollination with 1 : 1 mixtures of incompatible pollen and finely ground sugars, prevented rejection of incompatible pollen by the stigma surface. Lower sugar concentrations or lower ratios of sugar to pollen were less effective. No specificity for overcoming incompatibility was observed among 18 simple sugars and related sugar compounds.

Key words: Hazelnut – *Corylus avellana* – Incompatibility – Overcoming – Sugar

Introduction

In natural plant populations, self- and cross-incompatibility is a widespread, efficient mechanism to promote genetic diversity by preventing inbreeding. However, in a hazelnut (filbert) breeding program, incompatibility precludes obtaining many favorable parental combinations. The genetics of the incompatibility alleles (S-alleles) has been elucidated by Thompson (1979). Incompatibility in hazelnut is determined sporophytically by a single S-gene with multiple alleles. Dominance and codominance may occur between S-alleles in the pollen, but only codominance occurs in the stigma (unpublished results). All clones investigated to date are self-incompatible, and many cross-incompatible combinations have been identified.

The distinction between an incompatible and a compatible pollination is readily discerned microscopically using the aniline blue fluorescence technique. (Thompson 1979). Incompatible pollen germinates abundantly on the stigmatic styles and produces short, non-penetrating tubes. These tubes fluoresce strongly, due to heavy callose formation, and terminate in a pronounced bulb (Fig. 1). Compatible pollen tubes penetrate the stylar tissue and within several hours reach the base of the style (Fig. 2). These tubes have characteristic fluorescing callose plugs, but the pollen tube walls fluoresce much less intensely than those of incompatible tubes. This technique provides a reliable assessment of compatibility. Concurrence between stylar squash results and seed formation has been found during 14 years of testing prospective parents for compatibility.

The self-incompatibility recognition mechanism between pollen and pistil is incompletely understood, but there is evidence that lectins or lectin-like compounds may be involved. Glycoproteins are among the substances binding to the stigma pellicle in *Silene* (Mattson et al. 1974), and held in the exine of the pollen grain of *Brassica* (Heslop-Harrison et al. 1974). The lectin Concanavalin A (Con A) has been observed to bind to the stigma pellicle or style of several species (Delbart et al. 1983; Heslop-Harrison 1976; Knox et al. 1976) and to stylar glycoproteins of *Prunus* (Mau et al. 1982) and of *Brassica* (Nishio and Hinata 1980, 1982). Con A also binds to pollen wall substances of grasses (Watson et al. 1974), and to the plasma membrane of the pollen grain in *Lilium* (Leibowitz and Southworth 1983). This lectin has also been shown to modify pollen-stigma interaction. Pretreatment of *Brassica* stigmas with Con A prevented germination of both compatible and incompatible pollen (Kerhoas et al. 1983), while pretreatment of *Secale* stigmas with Con A inhibited penetration but not germination of compatible pollen (Heslop-Harrison and Heslop-Harrison 1981). Heslop-Harrison and Heslop-Harrison (1982) have proposed an incompatibility system for grasses based on a lectin or lectin-like recognition molecule.

The binding site of lectins may be masked by application of specific simple sugars, called hapten inhibition (Sharon and Lis 1972). If lectins are responsible for the highly specific cell recognition phenomenon of in-

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Fig. 1. Pollen tube growth in hazelnut styles after incompatible pollination ('Ennis'×'Montebello'). Note numerous strongly fluorescing short tubes terminating in a bulb

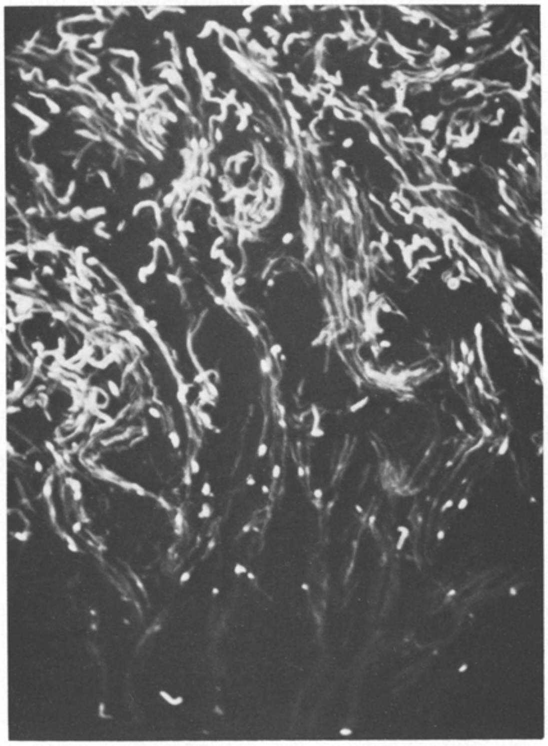

Fig. 2. Pollen tube growth in hazelnut styles after compatible pollination ('Ennis'×'Tonda di Giffoni'). Note many long tubes with callose plugs

compatibility in hazelnut, the inhibition of pollen tube penetration might be blocked by applying the appropriate sugar to the site of stigma-pollen interaction. We wished to investigate the possibility of overcoming incompatibility in hazelnut by the application of sugars to the site of pollen rejection, and to determine whether or not specific interactions exist between S-alleles and any of 18 sugars and sugar compounds.

Materials and methods

Detached pistillate flower clusters were held on moist filter paper in petri dishes at 5 °C for no longer than 24 h before pollination. Intact clusters, each consisting of 8–24 stigmatic styles, were treated with the appropriate pollen, sugar solution, or pollen-sugar mixture, and placed on moist filter paper in a petri dish at 20 °C. Pollen tube growth was evaluated after 12–15 h, at which time compatible tubes had approached the base of the style. Individual styles were separated from the cluster, squashed in decolorized aniline blue, and observed immediately with a fluorescence microscope (Thompson 1979).

Sugars were applied to the stigma either in solution or as a mixture of pollen and dry, finely ground sugar. Each sugar treatment involved six to eight pistillate clusters, averaging 16 stigmatic styles per cluster.

Experiment 1. The effect of sugar solution concentration

To determine the effect of different sugar concentrations on incompatibility, 'Lansing' and 'Kruse' flower clusters were immersed in 0.5 M, 1 M or 2 M solutions of 12 sugars for one min, allowed to dry, then self-pollinated. Sugars included pentose monosaccharides (D-arabinose, D-ribose, and D-xylose), hexoses (D-fructose, L-fucose, D-glucose, D-mannose, and L-rhamnose), disaccharides (sucrose and maltose), the sugar alcohol D-mannitol, and the amino sugar N-acetyl glucosamine. Untreated controls were pollinated with compatible and incompatible pollen to assess flower quality, pollen viability, and compatibility.

Experiment 2. The effect of the ratio of dry sugar to pollen

Four different ratios of ground dry sugars to pollen were applied to pistillate clusters of 'Montebello', 'Tonda di Giffoni', and '18–9'. Styles were dipped into mixtures of finely ground sugars (D-galactose, D-glucose, D-mannose, and D-xylose) and incompatible pollen at 1:1, 1:2, 1:4, and 1:8 by weight. Mixtures of dry sugars and compatible pollen were also applied to styles to determine if sugar would adversely affect compatible pollen germination and tube growth. Untreated compatible and incompatible pollens were applied to control flowers.

Experiment 3. The effect of rinsing pistillate clusters in water after sugar treatments

'Ennis' pistillate clusters were immersed in 1 M solutions of sucrose, D-glucose, D-galactose, D-xylose, D-mannitol, and D-mannose for 1 min. Clusters were then placed in a humid chamber for either 1 min or for 1 h before rinsing 15 s with tap water. Incompatible pollen was applied as soon as clusters appeared dry.

Experiment 4. Comparison of sugar solutions with dry sugars

Two methods of applying sugars were investigated in the laboratory to determine the most efficient and effective method of overcoming incompatibility in the orchard. Using a small brush, clusters of 'Jemtegaard 5', 'Lansing', and 'Ennis' were pollinated with a mixture of finely ground sucrose and incompatible pollen in ratios of 1 : 1 and 1 : 2 by weight. Alternatively, clusters were sprayed with 1 M and 2 M sucrose solutions, allowed to dry, and pollinated with incompatible pollen. Control clusters were incompatibly pollinated without sugar.

Experiment 5. The interaction of specific sugars and S-alleles

To determine whether specific interactions between sugars and S-alleles exist, pistillate clusters of six cultivars representing different S-alleles were pollinated with sugar-incompatible pollen mixtures or pretreated with sugar solutions prior to pollination with incompatible pollen. The cultivars and their S-alleles were 'Montebello' (S_1S_2)×'23–17' (S_1S_{12}) 'Negret' ($S_{10}S_?$) ×'W4–6' ($S_{10}S_{21}$), 'Ennis' (S_1S_{11})×'Montebello', 'Fitzgerald 20' (S_2S_{11}) selfed, 'Lansing' (S_1S_3) selfed, and 'Kruse' (S_1S_2) selfed. Sugars used included L-arabinose, D-arabinose, D-fructose, L-fucose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, N-acetyl glucosamine, L-rhamnose, D-ribose, sucrose, and xylose. In addition, maltose, lactose, sorbitol and inositol were tested with 'Ennis' (S_1S_{11})×'Montebello' (S_1S_2).

Table 1. The effect of three concentrations of 13 sugars on self-incompatibility in 'Lansing' and 'Kruse' hazelnut cultivars

Sugar	Concentration (M)					
	'Lansing'			'Kruse'		
	0.5	1.0	2.0	0.5	1.0	2.0
Compatible control	+	+	+	+	+	+
Incompatible control	–	–	–	–	–	–
D-arabinose	–	+	+	±	+	+
D-fructose	–	+	+	±	+	+
L-fructose	+	+	+	±	+	+
D-glucose	–	+	+	±	+	+
maltose	–	+	+	ND	ND	ND
D-mannitol	ND	ND	ND	±	+	+
D-mannose	+	+	+	±	+	+
N-acetyl glucosamine	±	+	+	±	+	+
L-rhamnose	–	+	+	±	+	+
D-ribose	–	+	+	ND	ND	ND
sucrose	+	+	+	±	+	+
D-xylose	–	+	+	–	+	+

+ Many long tubes, similar to those of a compatible cross
– Many short tubes, similar to those of an incompatible cross
± Mixed reaction; long tubes in some styles and short tubes in others
ND = Not done

Results

Immersion of 'Lansing' and 'Kruse' pistillate flower clusters in 1 and 2 M solutions of 12 sugars overcame pollen rejection on the stigma surface. Incompatible pollination of sugar-treated clusters resulted in pollen germination and tube growth similar to that of compatible pollinations (Table 1). Results were less consistent with 0.5 M solutions; pollen tube growth appeared compatible in some styles whereas in others it appeared incompatible.

Dipping styles of 'Montebello', 'Tonda di Giffoni', and '18–9' into dry sugar-incompatible pollen mixtures was, at the highest ratio (1 : 1), effective in overcoming the stigmatic barrier (Table 2). As was true of the lower sugar solution concentrations, the lower ratios of sugar-pollen mixtures (1 : 2, 1 : 4 and 1 : 8) were less consistent in their effect. Normal, long tubes were observed when mixtures of compatible pollen and sugars were applied, indicating no adverse effect of sugars on pollen germination or growth in the styles.

'Ennis' styles which were dipped in 1 M solutions of each of six sugars, rinsed with water 1 min later, and incompatibly pollinated supported typical incompatible tube growth. However, when rinsing was delayed for 1 hour, many long tubes resembling those of a compatible pollination were observed in the styles.

Application of a mixture of dry sucrose and incompatible pollen (1 : 1 and 1 : 2) by brush to stigmas

Table 2. The effect of different ratios of dry sugar-incompatible pollen mixtures on incompatibility in three hazelnut cultivars

	'Montebello' self				'18–9' × 'Montebello'				'Tonda di Giffoni' × '20–58'			
Compatible control	+				+				+			
Incompatible control	–				–				–			
	Ratio of sugar : pollen											
	1 : 1	1 : 2	1 : 4	1 : 8	1 : 1	1 : 2	1 : 4	1 : 8	1 : 1	1 : 2	1 : 4	1 : 8
D-galactose	+	+	+	+	+	+	±	–	+	–	–	–
D-glucose	+	+	±	–	+	–	–	–	+	+	–	–
D-mannose	+	+	±	–	+	+	±	–	+	+	–	–
D-xylose	+	+	+	+	+	+	±	–	+	+	±	–

+ Many long tubes, similar to those of a compatible cross
– Many short tubes, similar to those of an incompatible cross
± Mixed reaction; long tubes in some styles and short tubes in others

and spraying 1 M and 2 M solutions on the styles before incompatible pollination were equally effective in this experiment in overcoming the stigmatic barrier. Brushing a 1 : 1 mixture of dry sucrose and incompatible pollen on clusters proved to be the most convenient method for field use.

In trials with the six cultivars and 14 sugars used, incompatible pollination of pistillate clusters following immersion in 1 M or 2 M solutions, or pollination with mixtures of dry sugars and incompatible pollen (1 : 1) resulted in pollen tube growth comparable to that of a compatible pollination. Similar results were achieved on 'Ennis' with 1 M solutions of four additional sugar compounds: maltose, lactose, sorbitol and inositol. Thus, there were no specific interactions between sugars and cultivars with different incompatibility alleles.

Discussion

Overcoming incompatibility in hazelnut with sugars has been achieved in laboratory tests as demonstrated by stylar squashes. The mechanism of overcoming incompatibility with sugars as observed in these experiments does not appear to involve hapten inhibition, as originally postulated. The fact that 18 different sugars blocked the incompatibility reaction on the stigma surface of several cultivars representing different S-alleles suggests that binding of specific sugars with S-allele specific receptors was not involved. Application of high concentrations of sugar (1 M or 2 M) might be expected to mask sugar specificities, but no specificity was evident in our trials involving 0.5 M solutions. Gaude et al. (1983) did not find hapten inhibition of the binding of *Brassica* pollen to rabbit erythrocytes by 0.1 M xylose, fucose, alpha-methyl mannopyranoside, mannitol, glucose, N-acetyl glucosamine, galacturonic acid, maltose, lactose, or L-arabinose.

We suggest two possible roles for sugars in overcoming incompatibility in hazelnut. First, the high sugar concentrations may have osmotically damaged or plasmolyzed the cells of the stigma surface, the site of rejection of incompatible tubes. Osmotic damage could explain the increased effectiveness of higher sugar concentrations or dry sugar-pollen mixtures. Rinsing the stigma surface one minute after application of sugar may have prevented or reduced the osmotic stress.

Kroes' (1973) enzyme theory of incompatibility suggests a nutritional role for endgenous sugars. He proposed that an incompatible pollen tube may lack a specific enzyme (determined by its S-allele) required to release a necessary nutrient (e.g.) a monosaccharide) from a specific nutrient-protein complex (determined by stylar S-alleles) present in the style. Incompatible pollen tubes fail to grow due to lack of a required nutrient. Preliminary results mentioned by Kroes (1973) may also have relevance to our results. Following treatment of *Petunia* styles with 60 °C water for 5 min, thus overcoming the incompatibility response, he found that concentrations of free glucose, fructose, and two unidentified sugars increased tenfold. He proposed that heat-released endogenous sugars may stimulate in vivo growth of incompatible pollen tubes to such an extent that they are indistinguishable from compatible tubes. We have observed that the incompatibility response in hazelnut can also be overcome by hot water treatment (data not presented). Following Kroes' (1973) theory, exogenous sugars may supply carbohydrates necessary for incompatible pollen tubes to penetrate the stigma surface and commence growth in the style. Although Kroes was dealing with the stylar pollen tube inhibition of gametophytic incompatibility, and our work was with the stigma surface response of sporophytic incompatibility, a similar nutritional mechanism may be in operation.

The single gene-mediated trigger that controls the ability of pollen tubes to penetrate and grow through the style has not yet been demonstrated. Thus, the exact mechanism controlling incompatibility, and the role of sugar in overcoming it, remains unknown. The technique of brushing a mixture of dry sugar and incompatible pollen (1 : 1) on pistillate flower clusters appears to have potential for effecting seed formation in self- and cross-incompatible hazelnut combinations. Orchard testing of this method is in progress.

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