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Effect of Hexane and Humidity on Self-Incompatibility in *Brassica oleracea*

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Summary. The effects of hexane, high humidity, flower age and temperature in overcoming the self-incompatibility of *Brassica oleracea* were studied using three plants, each of which was homozygous for a different dominant S-allele. Hexane had a significant effect in all cases, but the size of the effect varied considerably. In one plant there was a marked interaction between the effect of hexane, humidity and flower age, but temperature had relatively little effect. In another plant high humidity alone gave a very much greater response than hexane alone. This plant gave as many self-seeds from the high humidity treatment as from bud selfing, indicating that the incompatibility reaction was almost completely overcome by the high humidity. The results are discussed in the light of current views of the mechanism of incompatibility in *Brassica.*

Key words: Self-incompatibility – *Brassica* – Humidity – Hexane – Flower Age

Introduction

The strength of the self-incompatibility reaction in *Brassica oleracea* is related to the *S*-allele involved. Plants with recessive *S*-alleles tend to have weaker self-incompatibility than those with dominant *S*-alleles (Thompson and Taylor 1966b). This paper deals solely with plants which have dominant S-alleles and are almost totally self-incompatible under normal circumstances. Even in such plants the self-incompatibility system is inoperative at the bud stage, as full seed set can be obtained by bud self-pollination (Kakizaki and Kasai 1933).

Bud-selfing is the method normally used to multiply the highly self-incompatible inbred lines used to make F_1 hybrid cultivars of *Brassica* crops. The production of inbred seed by this method is very expensive because of the hand labour involved (Johnson et al. 1970). A method which would completely break down the self-incompatibility at the open flower stage could greatly reduce the cost of inbred seed.

Several factors have been reported to affect the partial breakdown of self-incompatibility in *Brassica.* The two chief intrinsic factors are flower age (El Murabaa 1957, Lawson and Williams 1976) and stage of flowering (Johnson 1971). Important extrinsic factors include high temperature (Ockendon 1973, Visser 1977), high humidity (Carter and McNeilly 1975, 1976) and high concentrations of carbon dioxide (Nakanishi and Hinata 1975). Other treatments which partially overcome the self-incompatibility reaction include electric shocks (Roggen et al. 1972) and local heating of the stigma surface (Roggen and van Dijk 1976).

There have been few reports of the use of solvents for overcoming self-incompatibility in the Cruciferae, but Tatebe (1968) and Roggen (1974) did achieve this to some extent. Tatebe (1968) applied very small amounts of ether or 10% aqueous potassium hydroxide to the stigmas of the Japanese radish. Larger amounts of these chemicals damaged the stigmas and reduced seed set. Roggen (1974) used three cultivated forms of *Brassica oleracea* and washed the pollen briefly with 20 drops of solvent. His most successful treatment for overcoming self-incompatibility was to wash pollen with a 1:1 acetone/water mixture, but this gave only 1.4 seeds per pod in a highly self-incompatible clone.

Willing and Pryor (1976) tested the effect of a number of organic solvents in overcoming interspecific incompatibility in *Populus.* n-Hexane was the most satisfactory solvent tested, and was more effective when applied to the stigma than to the pollen. In testing the possible effect of hexane in overcoming self-incompatibility in *Brassica,* it was essential to consider the interactions between hexane and other factors which might be involved and to obtain some estimate of the magnitude of the effect which could be attributed to the hexane treatment. The main treatments studied were the effect of hexane and high humidity, but the effects of temperature and flower age were also investigated.

Materials and Methods

The plants used were homozygous for the *S*-alleles S23, 24 and 39. These *S*-alleles are high in the dominance series (Thompson and Taylor 1966a, van Hal unpublished) and normally confer very strong self-incompatibility. The plants were obtained by bud-crossing two different plants homozygous for the same *S*-allele. This gives plants more vigorous than the inbred parents from which they were derived, and ones which produce large numbers of flowers, an important attribute when studying the effect of flower age. The S23 and S24 plants were hybrids between marrow stem kale (*Brassica oleracea* var *acephala*) and Brussels sprouts (*B. oleracea* var *gemmifera*); the S39 plants were hybrids between two different Brussels sprouts plants.

The plants were grown in pots and during flowering (October and November 1976) were kept in an insect-proofed glasshouse with artificial illumination. The temperature was maintained as closely as possible between the range of 13-22°C.

The degree of self-incompatibility was assessed both by counting the number of pollen tubes in the styles 24 h after pollination and by assessing seed set 8-10 weeks after pollination. The pollen tube method is quicker and easier, because the flowers can be detached from the plant and kept in incubators at various temperatures, if desired. However, from the practical point of view, it is the effect of a treatment on seed set which is important. In two experiments both methods of assessment were used on the same set of flowers. In order to measure both pollen tubes and seed set in a single carpel, the style was cut cleanly at its base 48 h after pollination. Ovaries developed normally after removal of the style and seed set from the resulting pods was as good as that from control carpels where the style was left intact. The only drawback to this technique is that pollen tubes are more difficult to count in styles which have been left for 48 h instead of 24 h before fixation.

Styles were prepared for pollen tube counts by fixation in Carnoy's fluid, softening with 1N NaOH and staining with decolorised Aniline Blue, prior to examination under a fluorescence microscope (Kho and Baër 1968).

In experiments in which the effect of flower age was studied, three categories of flowers were used. Young flowers were those which opened on the day when the pollinations were made, intermediate flowers opened the day before, and old flowers, two days before.

No precise control of humidity was attempted. The pedicels of detached flowers were inserted through holes in the tops of small plastic boxes filled with water. The small boxes were enclosed in a large plastic box and because of water spillage from the small boxes, the atmosphere surrounding the flowers would have been almost saturated. Flowers on plants in the glasshouse were either left unbagged after pollination (low humidity) or were enclosed in a plastic bag into which a few drops of water were shaken (high humidity). The humidity in the glasshouse was 65-75% RH for most of the time, but fell to 50% RH for short periods during the day.

In a preliminary experiment, hexane was applied to the stigma surface using fine camel hair brushes (Willing and Pryor 1976), but it was difficult to control the amount of hexane applied in this way. In some styles so treated, unusually large amounts of callose were seen, suggesting damage caused by excessive amounts of hexane. A more satisfactory method was to use micro-pipettes containing 1 µl of hexane. By touching the micropipette gently on the stigma surface, it was possible to apply 0.2-0.3 µl of hexane per stigma, and no damage was seen in the style.

Results

In the experiments listed in Table 1 hexane had a significant effect in all cases. Flowers treated with hexane had a mean of 34.6 pollen tubes/style after self-pollination, while untreated flowers had a mean of 3.3 pollen tubes. The effect of hexane was least in experiments E and F, the only experiments in which the flowers were left attached to the plant. In experiment F, a higher humidity was obtained than in experiment E by enclosing the flowers in a polythene bag, but this had little effect on tube numbers. The effect of hexane was greatest in experiments A and G where the incompatibility reaction was almost completely overcome by this treatment. Experiment B was essentially a repeat of experiment A, performed 12 days later. A comparison of the results shows the variation in the effects of the same treatments imposed on different occasions. The higher temperature used

Table 1. effect of hexane, flower age and temperature on self-incompatibility. Each figure is the mean number of pollen tubes per style for six flowers

			Control			Hexane		
				Flower Age			Flower Age	
Experiment	Plant	Temperature	Young	Intermediate	Old	Young	Intermediate	Old
A	S23	20°C	0.0	9.2	29.3	70.0	65.0	52.5
B	S23	20°C	0.3	4.3	5.7	14.5	33.3	51.7
C	S23	25°C	2.5	7.0	10.8	13.0	34.7	55.5
D	S23	11-21°C	0.2	1.5	4.8	20.5	23.8	37.8
E	S23	13-25°C	0	0	0	0.8	0.7	17.2
F	S23	13-22°C	0	0	–	14.0	17.0	3.3
G	S24	20°C	0	1.0	4.7	63.3	60.0	76.7

Table 2. Number of pollen tubes per style compared with number of seeds per pod after self-pollination. Each figure is the mean for six flowers. All the flowers were on a single S39.39 plant

	Young Flowers		Intermediate Flowers		Old Flowers	
Treatment	Pollen tubes	Seeds	Pollen tubes	Seeds	Pollen tubes	Seeds
Unbagged control	0.0	0.0	0.3	0.0	2.5	0.0
Bagged control	41.8	14.0	75.0	11.0	75.0	9.5
Unbagged hexane	0.5	0.0	2.3	0.7	5.5	1.2
Bagged hexane	63.3	13.0	73.3	11.0	70.0	8.7

Table 3. Number of pollen tubes per style compared with number of seeds per pod after self-pollination. Each figure is the mean for six flowers. All the flowers were on a single S23.23 plant

	Young Flowers		Intermediate Flowers		Old Flowers	
Treatment	Pollen tubes	Seeds	Pollen tubes	Seeds	Pollen tubes	Seeds
Unbagged control	0	0	0.2	0	2.5	0.3
Bagged control	0.8	1.8	3.2	4.8	7.8	11.0
Unbagged hexane	0.3	0.2	0.8	0.3	9.0	2.5
Bagged hexane	7.6	8.7	8.4	9.4	31.4	17.0

in experiment C did not increase the number of tubes in comparison with experiment A, but the somewhat lower temperatures in experiment D may have been associated with the slightly lower tube numbers obtained. Overall, old flowers had a mean of 24.1 tubes, intermediate flowers 18.5 tubes and young flowers 14.2 tubes (LSD = 4.0 at $p = 0.05$), but the magnitude of the flower age effect varied from experiment to experiment. In experiments B and C, hexane had a much greater effect on old flowers than young flowers, suggesting an apparent interaction between these factors.

To assess the effect of hexane and humidity on seed set, flowers of an S39 plant were treated with hexane and either unbagged (low humidity) or bagged (high humidity). The effect of flower age was slight, as also was the low humidity treatment with hexane (Table 2). The high humidity treatment without hexane gave a high seed set, as did the high humidity plus hexane treatment. With this plant, hexane alone had little effect while high humidity almost completely overcame the incompatibility reaction.

A similar series of tests was carried out with plant S23 (Table 3) in which flower age, the hexane and the humidity treatments all had some effect. The maximum number of seeds was obtained with old flowers which were bagged and treated with hexane, indicating an additive effect of these three factors. This contrasts with the results obtained with S39 (Table 2), where humidity was the overriding factor and the other two were relatively unimportant. In a few cases in Table 3, more seeds per flower were recorded than pollen tubes per flower. The pollen tubes were not very distinct in this experiment because the styles were left for 48 h before being collected. A few tubes were missed when the counts were made.

The effect of high humidity in breaking down self-incompatibility in the S39 plant was compared with other possible methods of producing self-seed. Good seed set was obtained by bud-pollination, and by pollination of open flowers which had been treated at high humidity with or without hexane (Table 4). There was no significant difference between these three treatments, but the hexane treatment at low humidity gave a much lower seed set.

In addition to studying the effects of hexane on the stigmatic surface, the effects of hexane on pollen were investigated. Pollen was washed three times, for three minutes each wash. The treated pollen was dried over silica gel in a desiccator and brushed onto compatible stigmas but no pollen tubes were produced, indicating that the pollen had been completely killed. Two further pollen samples

Table 4. Comparison of four methods of obtaining self-seeds in an S39.39 Plant. Figures signify mean seeds per pod

	Treatment			
Flower Stage	Humidity	Hexane	Number of pods	Mean
Bud	Low	No	19	13.4 ± 1.33
Open flower	High	No	21	17.4 ± 1.26
Open flower	Low	Yes	21	2.9 ± 0.61
Open flower	High	Yes	21	15.9 ± 0.74

were treated: both the pollen and the hexane were thoroughly dried before being mixed together. Again the pollen was completely killed. This result contrasts with those of Iwanami and Nakamura (1972) who found that the viability of *Lilium, Camellia* and *Impatiens* pollen was not reduced by storing the pollen in a number of organic solvents, and that the only solvents which killed the pollen were alcohols. Furthermore, Willing and Pryor (1976) found that hexane did not reduce the viability of *Populus* pollen.

Discussion

Although the hexane treatment had a significant effect, the size of this effect varied considerably according to the circumstances, and was seldom great enough to substantially overcome the incompatibility reaction. Furthermore there was an apparent interaction between the hexane treatment, humidity and flower age. A similar interaction of factors affecting the degree of self-incompatibility was found by Johnson (1971).

The high humidity treatment drew a much greater response from the S39 plant than the hexane treatment, but this effect was far less evident with the S23 plant. This suggests the possibility of a genotype-environment interaction. Carter and McNeilly (1975, 1976) found that one of the two inbred lines of Brussels sprouts which they tested responded to high humidity much more than the other. Hexane treatment might have been more effective in overcoming the self-incompatibility reaction if larger quantities could have been used without damaging the stigma, but it is unlikely to be a satisfactory alternative to bud-pollination for the production of self-seed. High humidity treatment is more promising as a practical method, but is apparently less effective with some lines than with others.

The physiology of self-incompatibility in Brassica is not well enough understood at the biochemical level (see Heslop-Harrison 1975 for review) to explain adequately the effects of hexane and humidity reported here. Roggen (1974, 1975) suggested that lipoproteins play an important part in the recognition phase of the incompatibility reaction which is thought to occur on the stigmatic surface. Hexane may dissolve or denature such lipoproteins and hence interfere with the recognition reaction. Christ (1959) showed that high humidity greatly increased the amount of pollen germination after self-pollination; it may also increase the rate of germination by enabling pollen grains to imbibe moisture directly from the atmosphere rather than from the stigmatic papillae. If the stigmatic side of the incompatibility reaction is activated only in the presence of pollen, any pollen grains which germinated particularly rapidly might be able to penetrate the stigmatic surface before the incompatibility reaction became fully activated. More evidence is needed on the sources from which germinating pollen grains imbibe moisture and the speed of hydration and germination at different humidities.

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