

# The Effect of Pollination and Ethylene on the Colour Change of the Banner Spot of *Lupinus albifrons* (Bentham) Flowers

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## ABSTRACT

In *Lupinus albifrons* flowers the banner spot of the standard is initially coloured white or pale yellow. Two to three days after reaching the stage of full flower opening, this banner spot develops a pinkish blush and is deep magenta after a further 24 h. The development of this pigmentation is accelerated by exposure to ethylene in a concentration- and time-dependent manner. Flowers with a pinkish banner spot produced the greatest amounts of ethylene and production was much lower in flowers which had either completed the colour change or in which the banner spot colour remained unchanged. Treatments such as stigma removal or pollination increased the rate of ethylene production. Dissection of the flowers showed that while the banner spot is changing colour there is no change in the rate of production of ethylene from the standard, i.e. from the banner spot or surrounding tissue. The major sites of production at this time are the keel and pistil.

Isolated flowers withered within 2 d of removal from the plant and therefore did not show any change in the colour of the banner spot unless exposed to ethylene. The increase in banner spot pigment was about fourfold when isolated flowers were exposed to ethylene ( $0.24 \mu\text{l l}^{-1}$ ); however, the increase was less than twofold when isolated standards were exposed to ethylene ( $0.27 \mu\text{l l}^{-1}$ ). Application of silver thiosulphate (STS) to intact isolated flowers, as a 1 h pulse prior to ethylene exposure, partially prevented the pigment accumulation, whilst a continuous supply of STS reduced the ethylene-induced colour change by approx. 50%. Low concentrations of cycloheximide (CHI) ( $0.01 \text{ mg ml}^{-1}$ ) reduced the accumulation of pigment in the banner spot of ethylene-treated flowers, and higher concentrations ( $1.0 \text{ mg ml}^{-1}$ ) completely prevented the ethylene-induced colour change.

Key words: Ethylene, flower senescence, *Lupinus albifrons*, pollination.

## INTRODUCTION

The senescence of many flowers has been shown to be influenced by pollination (Halevy, 1986). Usually, pollination accelerates the pattern of senescence observed in unpollinated flowers. For example, pollination causes the wilting of carnation (Nichols, 1977) or *Petunia* flowers (Gilissen, 1977) or the abscission of the corolla in *Digitalis* (Stead and Moore, 1979). In each of these species, ethylene production increases following pollination. Furthermore, exposure to exogenous ethylene mimics the effect of pollination by causing wilting of *Vanda* orchids (Burg and Dijkman, 1967), carnations (Nichols, 1968) or *Petunia*

(Whitehead, Halevy and Reid, 1984) or by accelerating abscission of the corolla in *Digitalis* (Stead and Moore, 1983).

Whilst species exhibiting either corolla wilting or abscission have received considerable attention, probably because of the horticultural implications (Woltering and van Doorn, 1988), those which show changes in the colour of all or part of the flower have been somewhat neglected. The occurrence of such colour changes does, however, appear to be widespread with examples from over 50 Angiosperm families (M. Weiss, pers. comm.). Subtle pigmentation changes after pollination in parts of the inflorescence of, for example, *Phalaenopsis*, after pollination were reported by Curtis (1943) and in *Lantana camara* by Mathur and Mohan Ram (1978) and Mohan Ram and Mathur (1984). In some *Cymbidium* blossoms, anthocyanins accumulate in the labellum after

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pollination or treatment with ethylene (Arditti, Hogan and Chadwick, 1973). However, in species other than orchids, the role of ethylene in pollination-induced colour change has not been studied.

In this study we have examined the accumulation of anthocyanins by flowers of *Lupinus albus* following pollination or treatment with ethylene, and also the production of ethylene, both by intact flowers and isolated parts of the flower, in order to determine the role of ethylene in the pollination-induced response seen in many species of *Lupinus* (Wainwright, 1978; Schaal and Leverich, 1980).

#### MATERIALS AND METHODS

Individual flowers were removed from the flowering spikes of *Lupinus albus* (Bentham) that were growing in the arboretum at the University of California, Davis, CA. Flowers were removed at specific developmental stages (Fig. 1), the youngest flowers had the keel and standard tightly folded with no colour development (I); older flowers were taken just as the standard commenced opening (II). The youngest (i.e. closest to the top of the spike) flower with a fully reflexed standard was taken as the third stage (III). The fourth stage was similar but taken from lower down the spike and was therefore older (IV). Occasionally there were younger flowers higher up the spike in which the banner spot had already started to change colour. Older flowers were removed when the banner spot was turning pink (V), and finally when the spot had undergone a complete change to magenta (VI). Older flowers were selected on the basis of the visual appearance of the standard, either partially or fully wilted (VII) or completely withered (VIII).

At the completion of each experiment, the banner spot was excised from the standard and placed in a minimal volume of methanol containing 1% (v/v) concentrated HCl. Storage, if necessary, was at 4 °C. A minimum of eight samples (each with either four or five banner spots) were taken for each treatment. Extraction of the material was also in acidic methanol and, after recording the volume of each extract, the absorbance was read on a Cary 210 spectrophotometer at the wavelength of maximum absorbance (535 nm). The major pigment content of the banner spot was identified by one- and two-way paper chromatography as cyanadin and contents calculated using the molar extinction coefficient for cyanadin ( $2.95 \times 10^4$ ; Harborne, 1967).

For the measurement of ethylene production, individual flowers were placed in small glass vials with the cut end of the pedicel in water, left for

1–2 h for any wound-induced ethylene production to diminish and then enclosed with rubber 'Sub-seals' and left at 20 °C under low light. After sufficient time for a detectable amount of ethylene to accumulate in each tube (usually 2 h) a 1 ml gas sample was removed and analyzed with a Varian gas chromatograph using an alumina column at 70 °C and a sensitivity to  $>0.01 \mu\text{l l}^{-1}$  ethylene. Ethylene production was calculated by reference to the peak height of known calibration standards. For the measurement of ethylene production from isolated flower parts, a similar procedure was used after first allowing the production of wound-induced ethylene to subside.

For measurement of the effect of exogenous ethylene, flowers were removed when the standard was fully unfolded but before there was any colour change of the banner spot. Flowers were rested on small vials with the pedicel immersed in water and placed inside tanks with a flow (approx.  $27 \text{ l h}^{-1}$ ) of either ethylene-free air or appropriate concentrations of ethylene. Initial experiments assessed the effect of exposure for 10 h to various concentrations of ethylene ( $0.1\text{--}2 \mu\text{l l}^{-1}$ ); other experiments assessed the effect of varying the length of exposure (0–24 h) to ethylene ( $0.27 \mu\text{l l}^{-1}$ ). In experiments using isolated standards, these were placed on damp filter paper and then placed inside the appropriate ethylene-containing environment.

Silver thiosulphate (STS, 4 mM), an inhibitor of ethylene action (Veen, 1979), was applied either as a pulse (1 h) or continuously. Cycloheximide (CHI), an inhibitor of protein synthesis, was applied continuously at concentrations between 0.01 and 1 mg ml<sup>-1</sup>. The effect of these inhibitors on the colour change of the banner spot was studied either in the presence or absence of ethylene ( $0.24 \mu\text{l l}^{-1}$ ).

#### RESULTS

During the normal development and senescence of flowers of *L. albus* the banner spot undergoes a dramatic colour change, from an initially white or pale yellow colour to a deep magenta (Fig. 1). The pigment content of the banner spot of young flowers, before any visible colour change, was  $137.0 \pm 54.1 \text{ ng mg}^{-1}$  ( $n = 10$ ). For flowers in which the spot had changed colour, the concentration of cyanadin was  $565.3 \pm 82.3 \text{ ng mg}^{-1}$  ( $n = 10$ ). The magnitude of the response varied little between flowers of the same plant but more widely between plants, probably because the initial colour and extent of the banner spot varied considerably between plants. Tagging of individual flowers was used to determine the length of time between the

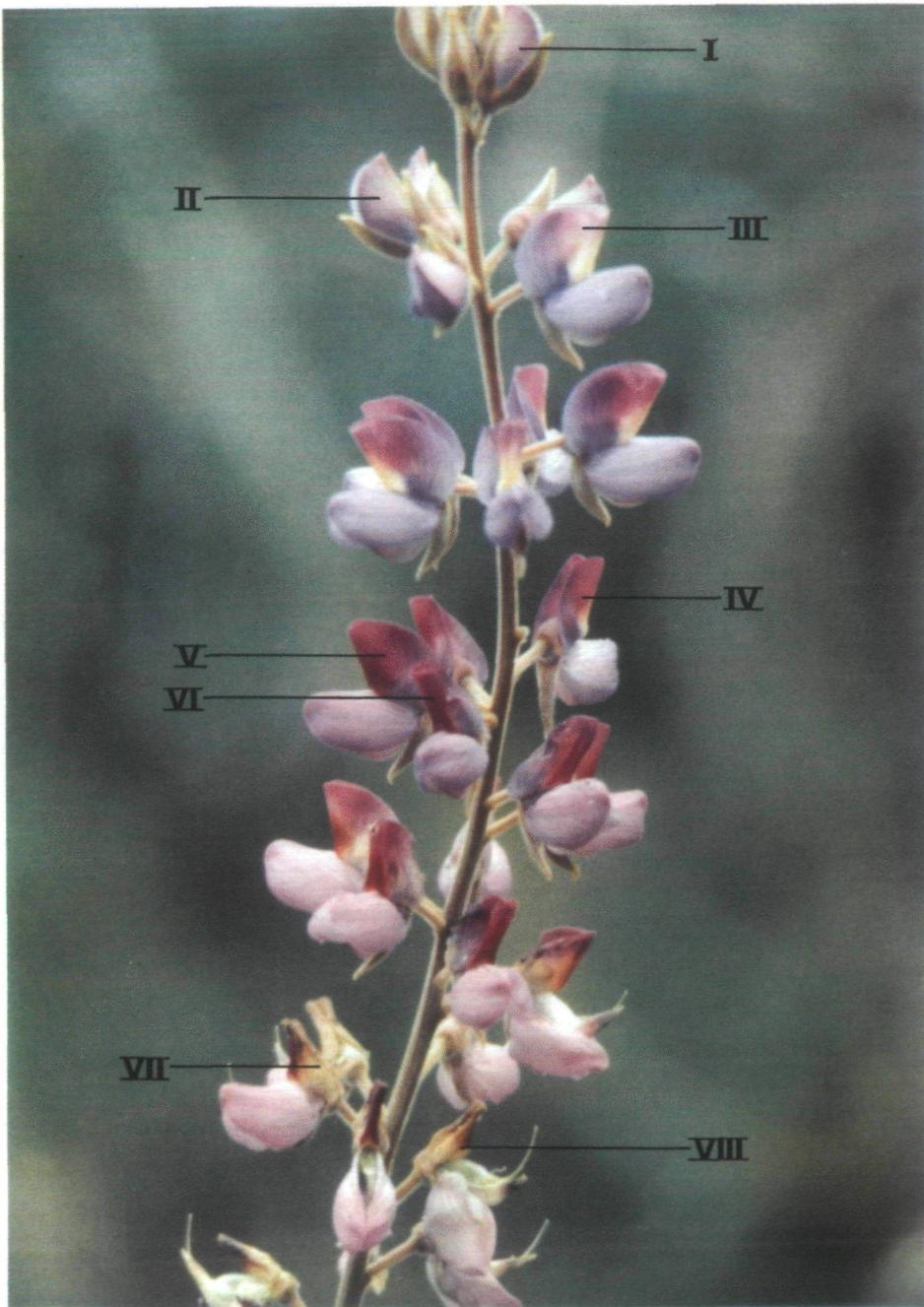


FIG. 1. A flowering spike of *L. albus*, with the various flowering stages indicated.

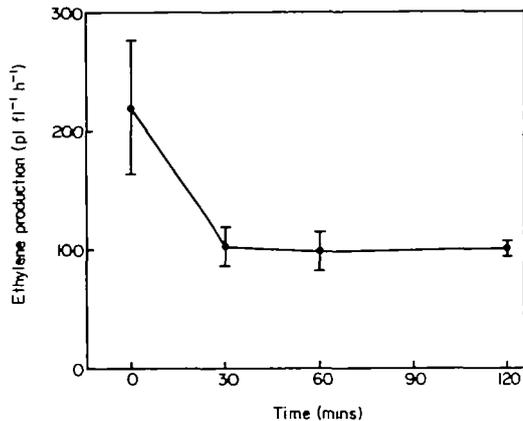


FIG. 2. Ethylene production from intact, isolated flowers of *L. albilfrons* at various times after removal from the flowering spike. Ethylene production was measured after 90 min enclosure and the data plotted as the mean ( $n = 22$ ) hourly rate of production ( $\pm$  s.e.) against the time of commencing enclosure.

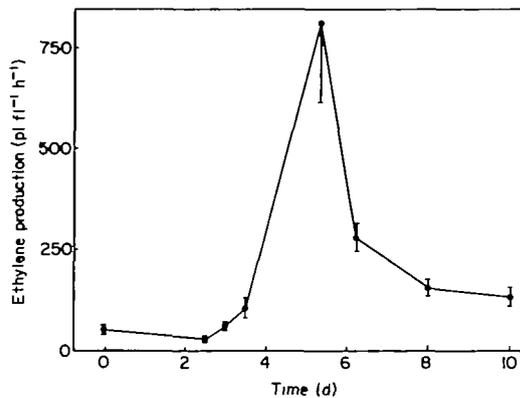


FIG. 3. Ethylene production by intact, isolated flowers of *L. albilfrons* at various stages of floral development; relative position of data points adjusted to account for the differences in time between each floral stage. The flowers were removed from open pollinated plants. Vertical bars represent standard errors.

identified floral stages. Thus, the standard started to unfold approximately 3 d after the first stage and this was complete within 1 d. The banner spot commenced its colour change about 2 d later and the colour change was complete within 1 d. The flowers persisted for a further 2 d with little sign of senescence. However wilting commenced at about this time, some 8 d after the first floral stage, and the flowers withered within 2 d.

Measurement of floral ethylene production immediately after removal of individual flowers from the plant showed that the initial burst of ethylene production due to wounding had subsided within 2 h (Fig. 2). After allowing wound-induced ethylene production to dissipate, the production of ethylene by the lupin flowers was relatively low for all stages except those in which the banner spot was either beginning to change, or had recently changed, colour (Fig. 3). Dissection of flowers with fully opened standards, but with varying degrees of banner spot pigment development, indicated that prior to any colour change the production of ethylene was similar from the

standard and the remainder of the flower. However, when anthocyanin was accumulating, ethylene production by the standard was unchanged whilst that of keel plus ovary, and the ovary alone, increased ten-fold. Therefore, the major sites of ethylene production at this stage did not include the standard (Table 1). Further dissection of the keel, ovary and style showed that the production from these tissues was approximately equal at the time of the banner spot colour change, even though in the younger flowers the ovary produced less than the keel (Table 1). Treatments such as pollination, stigma removal or stigma squeezing, all resulted in increased production of ethylene by the flowers (Table 2).

In response to exogenous ethylene, the banner spot of isolated flowers changed colour to that usually associated with the natural development/senescence of the intact flower. The response was concentration dependent, with a significant accumulation of pigment occurring in response to the lowest concentration of ethylene ( $0.05 \mu\text{l l}^{-1}$ ) (Fig. 4). When exposed to  $0.24 \mu\text{l l}^{-1}$  ethylene there was no detectable colour change after 6 h but after

TABLE 1. The production of ethylene (pl flower<sup>-1</sup> h<sup>-1</sup>) by intact flowers and dissected parts of flowers of *L. albilfrons* at various stages of colour development of the banner spot. Values are the means of 20 replicate flowers  $\pm$  s.e.

Banner spot colour	Intact flower	Standard	Keel + pistil	Pistil
Yellow	331.1 $\pm$ 62.4	173.5 $\pm$ 26.2	167.6 $\pm$ 29.0	47.2 $\pm$ 6.5
Pink	1383.2 $\pm$ 385.8	139.5 $\pm$ 22.9	1109.8 $\pm$ 359.2	450.8 $\pm$ 143.0
Red	288.6 $\pm$ 35.2	38.0 $\pm$ 4.3	428.0 $\pm$ 161.5	85.6 $\pm$ 37.9

TABLE 2. The effect of pollination, stigma damage or style removal on the production of ethylene ( $\mu\text{l flower}^{-1} \text{h}^{-1}$ ) from isolated flowers of *L. albilfrons* at differing times after treatment. All flowers were fully open but taken prior to any visible change in the banner spot colour. Values are the means of 16 replicate flowers  $\pm$  s.e.

Treatment	Time after treatment		
	2 h	20 h	44 h
Control	40.6 $\pm$ 5.8	20.9 $\pm$ 3.6	203.8 $\pm$ 21.5
Pollinated	459.2 $\pm$ 85.9	123.9 $\pm$ 13.1	191.4 $\pm$ 26.1
Stigma squeezed	445.9 $\pm$ 54.8	337.9 $\pm$ 83.5	581.8 $\pm$ 82.0
Style removed	838.9 $\pm$ 119.9	245.7 $\pm$ 48.0	252.2 $\pm$ 50.4

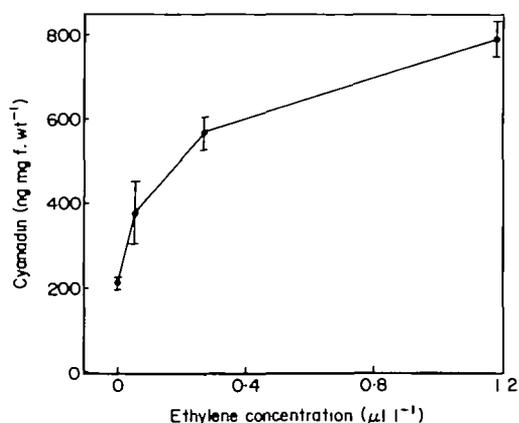


FIG. 4. The effect of a 10 h exposure to varying concentrations of exogenous ethylene on the accumulation of pigment in the banner spot of *L. albilfrons* flowers removed from the plant soon after full flower opening and therefore prior to pollination. Values are the mean of 11 flowers  $\pm$  s.e.

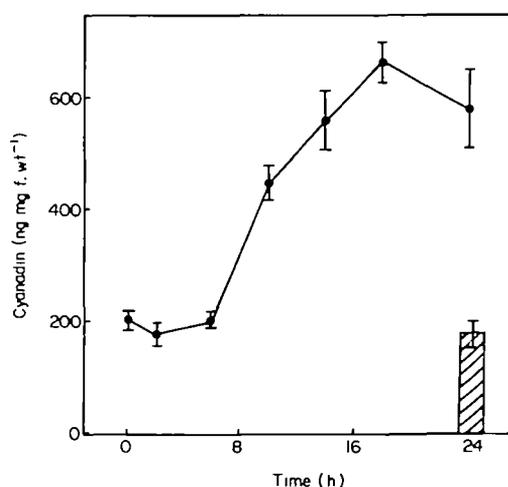


FIG. 5. The effect of various lengths of exposure to  $0.27 \mu\text{l l}^{-1}$  ethylene on the accumulation of pigment in the banner spot of flowers similar to those used in Fig. 4. The concentration of pigment in flowers treated with ethylene-free air for 24 h is shown by the hatched bar. Values are the mean of 11 flowers  $\pm$  s.e.

10 h, the pigment concentration was more than double that of the untreated controls (Fig. 5). The banner spot of isolated standards responds similarly to the intact isolated flower, although the magnitude of the response to exogenous ethylene was greatly reduced (Table 3).

The use of STS, an inhibitor of ethylene action, revealed that a short pulse (1 h) was sufficient to reduce the colour development that was always seen in water-treated controls upon exposure to ethylene (Table 4). Continuous STS treatment, throughout the exposure to ethylene was more effective than the pulse treatment at inhibiting the development of the pigmentation (Table 4). Experiments in which the effect of STS was investigated in the absence of applied ethylene were inconclusive because flowers held in water rarely showed any colour change; the flowers withered within 2 d of removal from the plant with

TABLE 3. The effect of exposure (10 h) to ethylene ( $0.27 \text{ nl ml}^{-1}$ ) on the colour development of the banner spot in isolated, but otherwise intact flowers, compared to detached standards. All flowers were fully open but taken prior to any visible change in the banner spot colour. Values are the means of six replicate groups each of four banner spots  $\pm$  s.e.

	Pigment concentration ( $\text{ng mg}^{-1}$ )	
	In air	In ethylene
Isolated standards	139.8 $\pm$ 39.0	207.2 $\pm$ 48.5
Intact flowers	129.4 $\pm$ 29.5	420.7 $\pm$ 46.8

TABLE 4. The effect of STS or cycloheximide on ethylene-dependant accumulation of pigment in the banner spot of isolated flowers. Ethylene exposure was for 16 h at  $0.24 \text{ nl ml}^{-1}$ . Values are the means of six groups each using five banner spots  $\pm$  s.e.

Treatment	Pigment concentration (ng $\text{mg}^{-1}$ )
Water	816.0 $\pm$ 67.2
STS (1-h pulse)	620.9 $\pm$ 86.0
STS (continuous)	454.9 $\pm$ 58.9
CHI (0.01 $\text{mg ml}^{-1}$ )	385.3 $\pm$ 38.5
CHI (0.1 $\text{mg ml}^{-1}$ )	239.9 $\pm$ 41.5
CHI (1 $\text{mg ml}^{-1}$ )	130.0 $\pm$ 21.5

TABLE 5. The effect of STS or cycloheximide on ethylene production from intact, isolated flowers of *L. albilfrons*. All measurements taken 12 h after treatment and, with the exception of those treated with an STS pulse, held in the treatment solutions throughout. Ethylene production measured as  $\text{pl flower}^{-1} \text{ h}^{-1}$  with a minimum of ten replicates per treatment

Treatment	Ethylene production ( $\text{pl flower}^{-1} \text{ h}^{-1}$ )
Water	83.0 $\pm$ 6.3
STS (1-h pulse)	117.6 $\pm$ 18.9
STS (continuous)	465.2 $\pm$ 113.4
CHI (0.01 $\text{mg ml}^{-1}$ )	155.4 $\pm$ 21.0
CHI (0.1 $\text{mg ml}^{-1}$ )	161.7 $\pm$ 26.3
CHI (1 $\text{mg ml}^{-1}$ )	100.8 $\pm$ 9.5

the banner spot remaining pale yellow. When left unpollinated on the plant however, several days elapsed between the flower opening fully and anthocyanin accumulation commencing.

Cycloheximide was more effective than STS at preventing the ethylene-induced colour change of the banner spots; concentrations as low as  $0.01 \text{ mg ml}^{-1}$  reduced the accumulation of pigment, whilst higher concentrations ( $1 \text{ mg ml}^{-1}$ ) completely prevented the ethylene-induced development of banner spot colouration (Table 4). Both STS and CHI treatment caused an increase in the rate of ethylene production, relative to water-treated flowers (Table 5).

#### DISCUSSION

The colour change of the banner spot of the flower of *L. albilfrons* is the result of a three- to fivefold increase in anthocyanins. The extent of the increase depends on the individual plant, since the initial

size and colour of the banner spots varies considerably from plant to plant. This situation also occurs in *Digitalis*, where the pigment content of the entire corolla is very variable (Stead and Moore, 1977). This colour change appears to be accelerated by pollination and probably deters further insect visits, therefore avoiding unnecessary wastage of pollen. Although the banner spot colour changes, individual flowers do not wilt or abscind for a further 2 d and the persistence of these flowers maximizes the overall attractiveness of the inflorescence to pollination vectors, a species of *Bombus* (Wainwright, 1978; Schaal and Leverich, 1980). Close observations of these flowers show that bees do not visit flowers once the banner spot has changed colour (data not shown). In other species, the response to pollination is very different. In *Digitalis*, the corollas abscind within 24 h of successful pollination (Stead and Moore, 1979), whilst in *Petunia* the corolla loses turgidity and wilts (Gilissen, 1977). Similarly, the characteristic petal in-rolling seen in senescent carnations is also accelerated by pollination (Nichols, 1977). Furthermore, in all of these species, the senescence of the flower is associated with increased ethylene production which occurs sooner in pollinated flowers. In each of these species the application of ethylene also causes premature flower senescence (Nichols, 1968; Stead and Moore, 1983; Whitehead *et al.*, 1984). The present study suggests that the colour change seen in *L. albilfrons* may also depend on ethylene, although in a manner that differs from other species.

The endogenous rate of ethylene production is comparatively low from floral buds or fully open flowers with pale banner spots, being less than  $100 \text{ pl flower}^{-1} \text{ h}^{-1}$ , or considerably less than  $1 \text{ nl g}^{-1} \text{ f. wt. h}^{-1}$ . However, in those flowers in which the banner spot can be seen to be changing, the rate is nearly tenfold higher. In *Digitalis*, at the time of corolla abscission, the increase in ethylene production is also about tenfold, although the rate of production prior to pollination is considerably greater than that recorded in *L. albilfrons* (Stead and Moore, 1983). In those flowers in which the colour change is complete, the rate is reduced to about  $150 \text{ pl flower}^{-1} \text{ h}^{-1}$ . In contrast to *Petunia* (Whitehead *et al.*, 1984) and *Dianthus* (Nichols, 1968), there is no evidence of a peak of ethylene production associated with petal wilting, even though the petals of *L. albilfrons* do wilt some 2 or 3 d later, after the banner spot has changed colour.

Banner spot colour change can be induced by application of ethylene; exposure to  $0.05 \mu\text{l l}^{-1}$  almost doubled pigment concentration, relative to flowers held in air, after only 10 h. Exposure to

higher concentrations caused a greater response, while  $1.2 \mu\text{l l}^{-1}$  for 10 h was sufficient to effect a complete colour change, i.e. a fourfold increase in pigment content. By choosing an intermediate concentration of  $0.27 \mu\text{l l}^{-1}$  ethylene, the colour change was followed over a 24-h period. There was no change in the pigment content of the banner spot of flowers held in ethylene-free air for 24 h while those in ethylene also showed no increase when held in ethylene for 6 h or less. However, after 10 h, the pigment content was significantly increased and colour development was complete after about 18 h exposure. At this time, the concentration of cyanadin was equal to that of flowers allowed to age naturally, either pollinated or unpollinated, on the plant.

The increase in ethylene production was derived almost entirely from the keel and ovary, as the rate of ethylene production from the standards declined slightly when the banner spot started to change colour. Furthermore, once the colour change was complete, the production of ethylene by the standard decreased significantly. Measurements of ethylene production from either the keel plus pistil or the pistil alone, showed that there was a tenfold increase in production from these tissues. The slight increase in the total ethylene produced from the dissected flower parts, relative to the intact flowers, is probably due to the persistence of wound-induced ethylene production. From these data it is clear that the increase in ethylene production, seen during the banner spot colour change, is entirely due to the increases seen from the keel and pistil and not from the standard. Moreover, whilst the pistil contributed nearly half of the ethylene produced by the keel and pistil together, the fresh weight of the pistil was approximately only one-tenth that of the keel: the rate of production from the pistil was therefore approximately  $100 \text{ nl g f. wt}^{-1} \text{ h}^{-1}$ . This situation is identical to other species studied in which the pistil, and in particular the style, has the highest rate of ethylene production per unit fresh weight of all the floral tissues (Hall and Forsyth, 1967; Nichols, 1977; Suttle and Kende, 1978). Pollination of isolated flowers resulted in a tenfold increase in the amount of ethylene produced 2–4 h following pollination: this rate of ethylene production however, is still only half that recorded from flowers at the time the banner spot commenced changing colour (Fig. 3). Similar increases occur when the stigma is squeezed but a larger increase is detected after style removal. Although the styler tissues undoubtedly possess a high capacity for ethylene production per unit weight, the tissue is very small when compared to the much larger ovary, and thus the removal of

this tissue has little effect on the ethylene production from the remainder of the flower. In fact, the higher rate of ethylene production recorded following style removal may be the result of increased tissue damage as removal of the style in these flowers is particularly difficult. In *Petunia* too, stigma squeezing and style removal both hasten corolla wilting and cause increased rates of ethylene production, with style removal again giving the greatest response (Lovell, Lovell and Nichols, 1987).

Whilst exogenous ethylene induces a colour change of the banner spot in intact flowers, albeit isolated from the plant, the response of isolated standards was considerably less marked. There was only a 50% increase in the banner spot pigment content of isolated standards, relative to air-treated controls, but more than a threefold increase in the pigment content of the same area of tissue when the isolated flowers remained intact. It would therefore seem that either the keel and/or pistil are a source of pigment precursor, or some other factor which is involved in the sensitivity of the banner spot to applied ethylene. Halevy, Whitehead and Kofranek (1984) reported that in *Cyclamen*, unpollinated flowers produce very little ethylene and show corolla discolouration and wilting. The senescence of such flowers is not accelerated by exogenous ethylene. The corollas of pollinated *Cyclamen* flowers do, however, abscise and this process is accompanied by a large increase in ethylene production. Furthermore, abscission can be hastened if the pollinated flowers are exposed to ethylene, thus pollination in some way renders these flowers sensitive to ethylene. In *Petunia*, the response of the corolla to applied ethylene is also dependent on a 'sensitivity' factor. Exposure to ethylene soon after pollination is less effective at accelerating senescence than application a few hours later and yet, in terms of the ethylene production the flowers are identical (Whitehead and Halevy, 1989). The identity of this sensitivity factor has yet to be proven although data of Whitehead and Halevy (1989) suggest that it may be a fatty acid. If a similar situation should exist in *L. albifrons* then the removal of the standard from the remainder of the flower could remove the source of such a sensitivity factor therefore rendering the banner spot less sensitive to applied ethylene.

In many situations a change of colour has been ascribed to changes in vacuolar pH since anthocyanin pigments are well known to be pH-sensitive. In *Lathyrus hirsutus*, the change in petal colour is accompanied by a rise in the pH of the expressed sap (Pecket, 1966). The cause of this pH change may be the breakdown of the tonoplast and the

mixing of the vacuolar and cytoplasmic compartments as has been suggested in *Ipomoea* and *Tradescantia* (Matile and Winkenbach, 1971; Suttle and Kende, 1978). There is, however, no evidence of the loss of membrane integrity in *Lupinus* as the flowers persist, without wilting, for two to three days after the colour change is complete. This, together with the observation of a lag in the accumulation of pigment in response to exogenous ethylene and the inhibition by cycloheximide, suggests that the pigment is synthesised *de novo* during the banner spot colour change.

In common with other studies (Yang and Riov, 1982) the effect of CHI was not due to a decrease in endogenous ethylene production, in fact the rate of production increased as the result of treatment with CHI (Table 5). As expected STS, a potent inhibitor of ethylene action (Veen, 1979), partially reversed the effect of exogenous ethylene, revealing that STS was probably acting at the cellular level to antagonise ethylene action. In many other systems where STS has been shown to overcome the effect of exogenous ethylene (Nichols and Kofranek, 1982; Hoyer, 1986; Joyce, Reid and Evans, 1990) it has also been found to reverse similar physiological changes associated with increased ethylene production, thus lending support to the view that the endogenous ethylene was a causal agent. Although we were unable to test STS on excised lupin flowers in the absence of applied ethylene because their life was too short, its activity in the presence of applied ethylene adds credence to the view that natural ethylene promotes the colour change of the lupin banner spot. Furthermore, no attempt was made to quantify the amount of silver taken up. In *Hibiscus* (Woodson, Hanchey and Chisholm, 1985), STS was only effective at preventing senescence if applied to immature petals and it may be that the transpiration by the mature flower, and therefore the uptake of solution, was such that insufficient silver was present at the site of ethylene action to totally prevent the effect of applied ethylene.

Pollination of isolated flowers also failed to cause banner spot colour change, possibly because the flowers wilted too soon. It was noticeable however, that the rate of ethylene production from these flowers never attained the level of production associated with flowers removed from the plant at the stage when the banner spot colour was changing, suggesting that the isolated flowers were either unable to maintain a comparable rate of ethylene production (to that achieved while remaining attached to the plant), or that pollination of isolated flowers was ineffectual. Certainly pollen

germination, and pollen tube growth, occurred but the rate was not compared to that achieved *in situ*.

The present study indicates that, in common with other species, pollination can hasten the ageing process of *L. albifrons* flowers and bring about the characteristic colour change in the banner spot. This change appears to be brought about by ethylene as the banner spot pigment accumulation is accelerated by ethylene and increased ethylene production occurs concomitantly with colour development. The site of synthesis of the increased ethylene production is however, not the banner spot nor the immediately adjacent tissues of the standard, but rather the keel and ovary. It therefore seems probable that the localised increase in ethylene production in these tissues induces the formation of an, as yet, unidentified pigment precursor which is then translocated to the banner spot where it contributes to the observed colour development.

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