Determinating herbicide damage to light harvesting apparatus of radishes (*Raphanus sativus*).

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Abstract

In vivo spectral analysis of radish leaves sought effects on radish light harvesting apparatus (LH) of the herbicides: clomazone (FMC57020; [2-(2-chlorophenyl) methyl-4,4 dimethyl-3-isoxalidinone), diuron (DCMU; [3-(3,4-dichlorophenyl)-1,1-dimethylurea)] and amitrol (*H*-1,2,4-triazole-3-amine). All herbicides used cause visual symptoms of chlorosis or damage chloroplast membranes and thus disrupt LH structure. The spectra were obtained at early stages of the chlorotic effect. The spectra was analyzed by the fourth derivative technique. The frequency of occurrence of fourth derivative maxima was analyzed statistically. Statistical analysis of peak frequency showed multiple and complex changes occurred in LH spectra in response to herbicide treatment. Amitrol effects were readily apparent and are those described here. The most prominent effect of amitrol was the narrowing and apparent red shift of LH component *Cb 670* (French C.S., Brown J.S. and Lawrence M.C., 1972. *Plant Physiol.*, **49**, 421-424.). Further analysis of *Cb 670* revealed fine structure consisting of at least three bands (*Cb 670a., Cb 670b* and *Cb670c*). The effects of amitrol on this system resulted in the loss of two fine structure bands (*Cb 670a, Cb 670c*). The result is that the remaining fine structure band (*Cb 670b*) dominates the spectral envelope of the parent band (*CB 670*) causing parent band narrowing and red shift. Since plant response to herbicides reveals the presence of these chemicals, this effect may be useful in herbicide detection.

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Table 1. Means (and standard deviations) of *in vivo* peaks of light harvesting components *Cb 649, Cb 660 and Cb 670* treated with amitrol, command and diuron. Variance (standard deviation^2) from all *Cb 670* data, except data from amitrol treated leaves, was pooled to determine if amitrol effects differed from effects of all other treatments. * *F* test significant at 0.05 in comparison with with pooled variance (Anderson, 1987 p.44). Fine structure determined through the probability plot technique as shown in Materials and Methods and Results and Discussion. Anova analysis significance at 0.99 level is indicated by superscript letters.

	Main bands (nm)		
	Cb 649	Cb 660	Cb 670
control	649.80 (0.33)	659.47 (3.73)	669.07 (2.08)
amitrol	649.49 (0.94)	660.46 (2.76)	669.11 (0.41)*
command	649.58 (0.40)	661.45 (3.37)	668.93 (1.60)
diuron	649.59 (0.74)	660.03 (2.68)	669.47 (1.59)

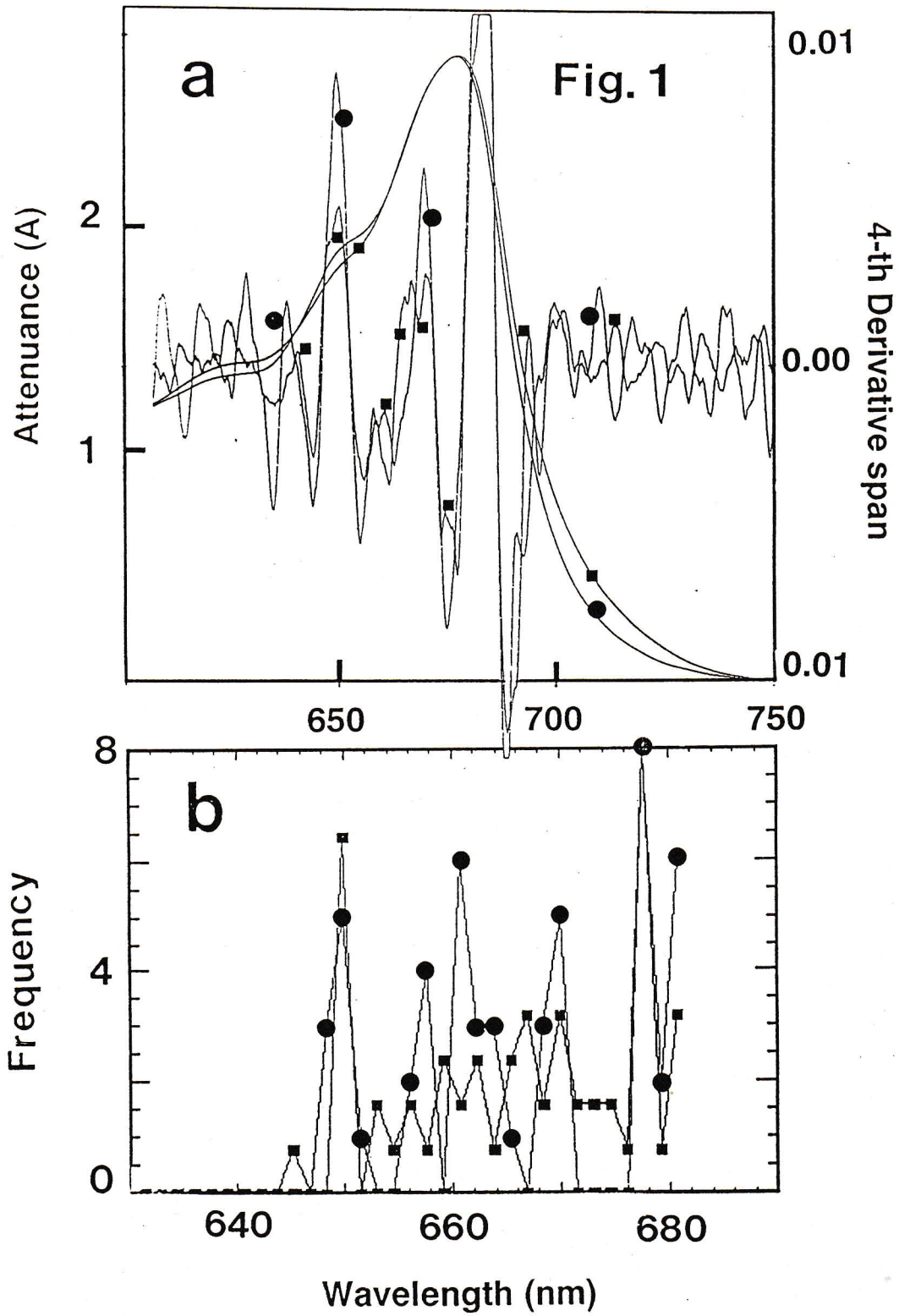
	Fine structure of Cb 670 (nm)		
	Cb670a	*Cb 670b*	*Cb 670c*
Pooled data	667.18 (0.60)^a	669.38 (0.57)^b	670.8 (0.07)^c
amitrol (from above)	none	669.47 (0.41)	none

Figure Legends

Figure 1. Spectral characteristics of normal (control) and amitrol treated leaves. 1a. Comparison of attenuance **in vivo** *spectra and fourth derivative analysis of control and amitrol treated leaves.* In both figures the closed circles indicate spectra from amitrol treatments, the closed squares spectra of controls. The less abruptly changing curves that originate at the lower right of the figure are the attenuance (~absorbance) spectra. The more abruptly changing traces that originate at the middle right of the figure are the fourth derivative traces. Figure 1b shows frequency of fourth derivative peaks from multiple spectra of control and amitrol treated leaves.

Figure 2. Fine structure of Cb 670. Figure 2a frequency plot of fourth derivative peak maxima from pooled spectra from clomazone, control, diuron treatments. The inset of Fig. 2a shows the three fine structure bands of *Cb 670* which are generated using a normal curve linearizing program. *Figure 2b* shows a continuous trace corresponding to normal curve that would be expected for a normal distribution of data points. The data points shown show that the curve is not homogenous, but shows fine structure. The inset of Fig. 2b shows the more linear distribution of *Cb 660* of the control which more closely represents that of a normal curve.

Figure 3. The upper part of the figure shows the frequency of fine structure *Cb 670b* from the pooled samples of clomazone, control and diuron treated *Cb 670*, generated from data shown in Fig. 2. The lower part of the figure shows the total frequency of fourth derivative peaks from *Cb 670* of amitrol treated leaves. Statistical analysis of this data is presented in Table 1.


a
Fig. 1
Attenuance (A)
2
1
0.01
0.00
0.01
4-th Derivative span
650
700
750
b
Frequency
8
4
0
640
660
680
Wavelength (nm)

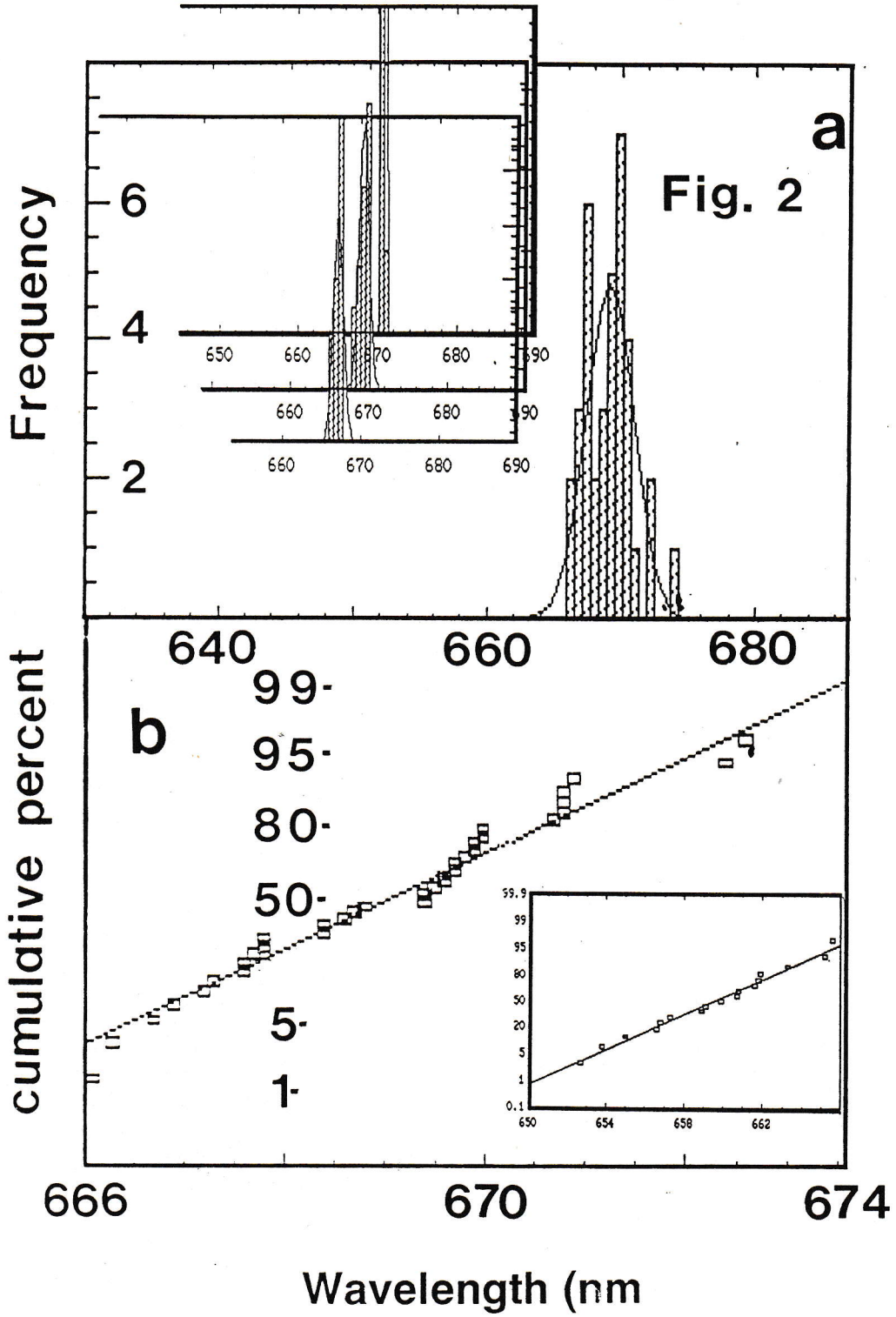

a
Frequency
6
4
2
650
660
670
680
690
640
660
680
b
cumulative percent
99
95
80
50
5
1
99.9
99
95
80
50
20
5
1
0.1
650
654
658
662
666
670
674
Wavelength (nm

Fig. 2

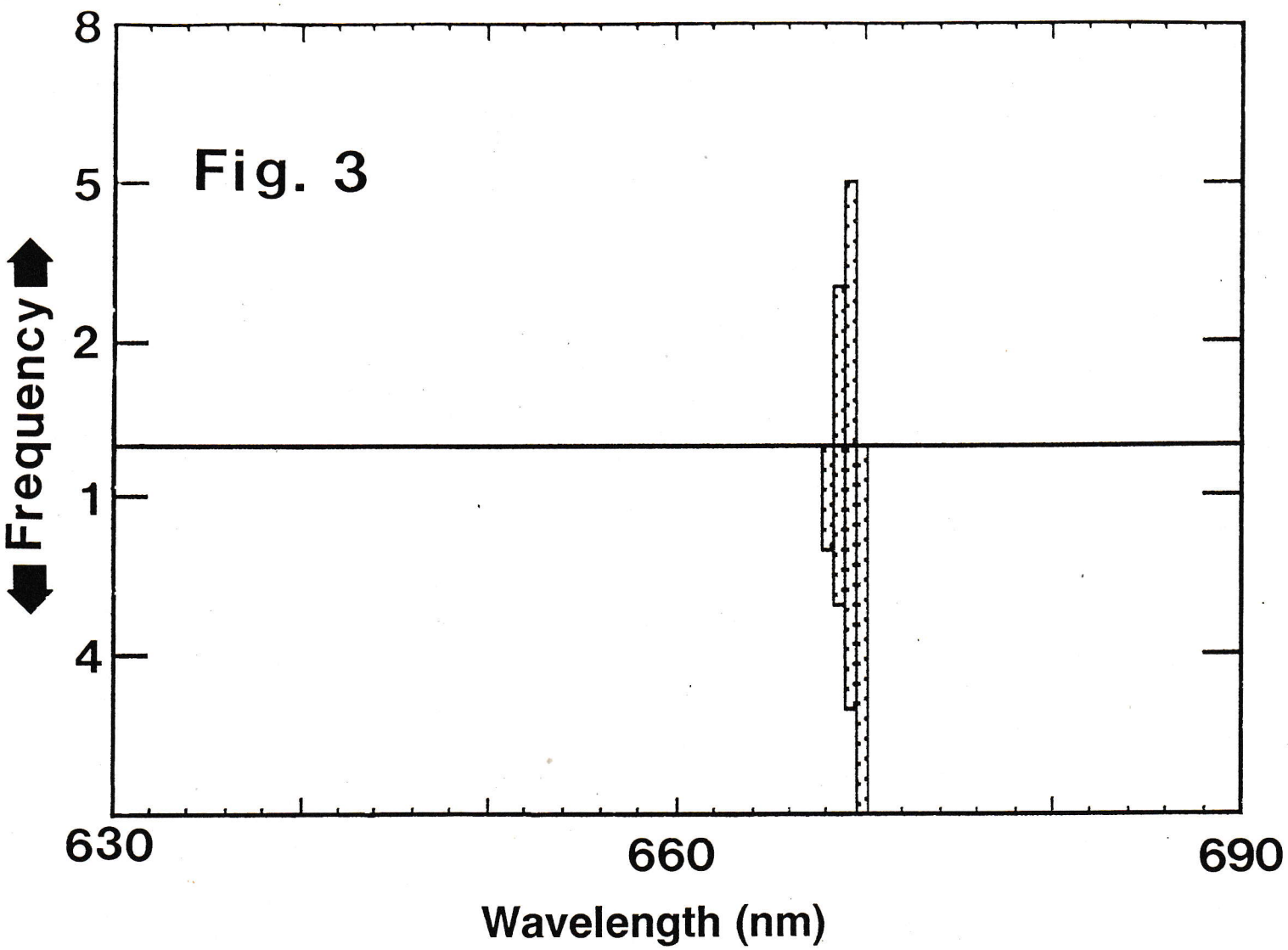


Fig. 3
8
5
2
1
4
Frequency
630
660
690
Wavelength (nm)

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Table 1. Means (and standard deviations) of *in vivo* peaks of light harvesting components *Cb 649, Cb 660 and Cb 670* treated with amitrol, command and diuron. Variance (standard deviation^2) from all *Cb 670* data, except data from amitrol treated leaves, was pooled to determine if amitrol effects differed from effects of all other treatments. * *F* test significant at 0.05 in comparison with with pooled variance (Anderson, 1987 p.44). Fine structure determined through the probability plot technique as shown in Materials and Methods and Results and Discussion. Anova analysis significance at 0.99 level is indicated by superscript letters.

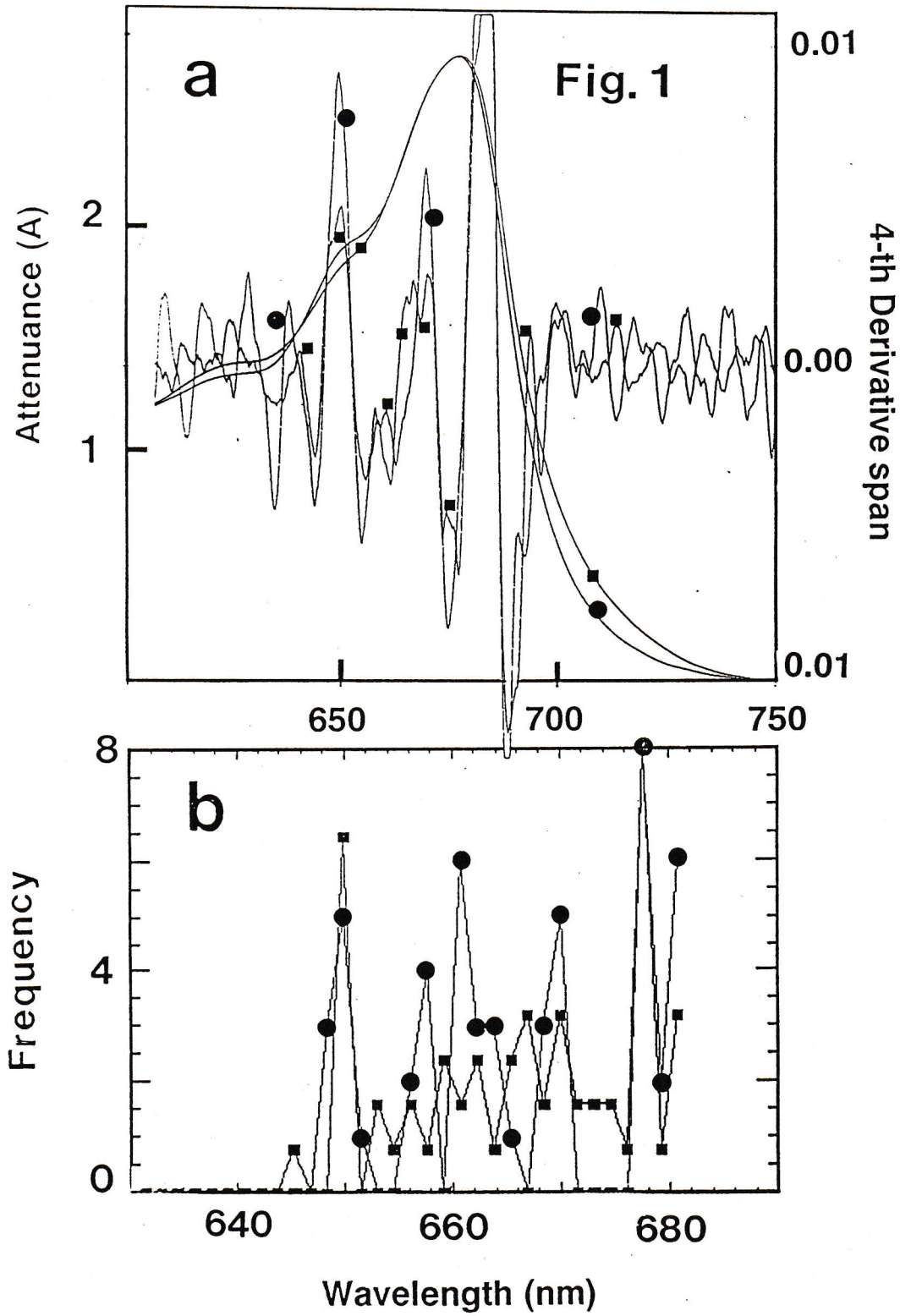
	Main bands (nm)		
	Cb 649	Cb 660	Cb 670
control	649.80 (0.33)	659.47 (3.73)	669.07 (2.08)
amitrol	649.49 (0.94)	660.46 (2.76)	669.11 (0.41)*
command	649.58 (0.40)	661.45 (3.37)	668.93 (1.60)
diuron	649.59 (0.74)	660.03 (2.68)	669.47 (1.59)
	Fine structure of Cb 670 (nm)		
	Cb670a	*Cb 670b*	*Cb 670c*
Pooled data	667.18 (0.60)^a	669.38 (0.57)^b	670.8 (0.07)^c
amitrol (from above)	none	669.47 (0.41)	none

Figure Legends

Figure 1. Spectral characteristics of normal (control) and amitrol treated leaves. 1a. Comparison of attenuance **in vivo** *spectra and fourth derivative analysis of control and amitrol treated leaves.* In both figures the closed circles indicate spectra from amitrol treatments, the closed squares spectra of controls. The less abruptly changing curves that originate at the lower right of the figure are the attenuance (~absorbance) spectra. The more abruptly changing traces that originate at the middle right of the figure are the fourth derivative traces. Figure 1b shows frequency of fourth derivative peaks from multiple spectra of control and amitrol treated leaves.

Figure 2. Fine structure of Cb 670. Figure 2a frequency plot of fourth derivative peak maxima from pooled spectra from clomazone, control, diuron treatments. The inset of Fig. 2a shows the three fine structure bands of *Cb 670* which are generated using a normal curve linearizing program. *Figure 2b* shows a continuous trace corresponding to normal curve that would be expected for a normal distribution of data points. The data points shown show that the curve is not homogenous, but shows fine structure. The inset of Fig. 2b shows the more linear distribution of *Cb 660* of the control which more closely represents that of a normal curve.

Figure 3. The upper part of the figure shows the frequency of fine structure *Cb 670b* from the pooled samples of clomazone, control and diuron treated *Cb 670*, generated from data shown in Fig. 2. The lower part of the figure shows the total frequency of fourth derivative peaks from *Cb 670* of amitrol treated leaves. Statistical analysis of this data is presented in Table 1.


a
Fig. 1
Attenuance (A)
2
1
0.01
0.00
0.01
4-th Derivative span
650
700
750
b
Frequency
8
4
0
640
660
680
Wavelength (nm)