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SASKATCHEWAN CANOLA
DEVELOPMENT COMMISSION

UV-B Radiation Affects Canola Yield

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A joint project between CANODEV, University of Guelph and Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre (ECORC)

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Introduction

The amount of UV-B radiation reaching the Earth's surface is increasing yearly. By the year 2005 to 2010, the amount of UV-B radiation bombarding our plants is estimated to be 20 % greater than it was in the mid 80's. UV-B radiation, in sufficient doses, harms sensitive plants and reduces yields. Preliminary research on the effects of UV-B radiation on canola revealed that both *Brassica napus* and *B. rapa* varieties were sensitive to elevated levels of UV-B radiation.

The best method for determining the effects of increased UV-B on canola is to grow the plants in the field and augment the radiation by suspending UV-B emitting bulbs over the canopy. This method is expensive, plot size is small and only a limited number of cultivars can be tested at one time. In 1996, a field trial at the University of Guelph UV-B screening facility revealed that canola was susceptible to increased UV-B radiation. With an increase in UV-B radiation equivalent to a 20 % decrease in the ozone layer *B. napus* and *B. rapa* varieties had their yield reduced by 15 and 14 %, respectively.

It may be possible to develop UV-B tolerance screening methods that correlate with yield reductions, thereby permitting more cultivars to be screened for UV-B tolerance These methods may then be incorporated into breeding programs. One possible method is to screen for the production of UV-B absorbing pigments called flavonoids which are produced in response to elevated levels of UV-B. When bombarded by UV-B photons flavonoids oxidize, thereby dissipating energy and protecting sensitive organelles.

To reduce the impact of increased UV-B on Canadian agriculture, tolerant varieties must be identified and the mechanisms of tolerance determined. We must determine if directed selection for UV-B tolerance is possible. We must determine if there is genetic variability for UV-B tolerance in our important economic species like canola.

Objectives:

- To screen 5 varieties of *B. napus* and 3 varieties of *B. rapa* for tolerance to UV-B radiation and determine if there is genetic diversity for UV-B tolerance in canola.
- To determine if UV-B absorbing pigment production is related to yield effects.

Methods:

The experiment was conducted at the Elora Research Station (43° 39'N, 80° 25'W, 376 m). The facility has 12 test areas, measuring 1.3 m X 3.6 m. A metal rectangular frame containing 12, UV-B fluorescent bulbs spaced 30 cm apart was suspended above each test area by a system of supporting posts and overhead wires. A pulley was used to change the height of the lamp frame and consequently the UV-B radiation dosage.

Each rectangular test area was further subdivided into eight plots, 1.3m x 0.34m. There were two rows per plot spaced 17.5 cm apart. Because of the difference in maturity between the *napus* and *rapa* cultivars, the species were kept in blocks separated by a guard row. Each test area had a block of 5 plots and a block of 3 plots. A guard row was planted at the extremities of each test area. Cultivars were assigned at random to a plot in each species block of the test area. Eight cultivars, five *B. napus* and three *B. rapa* of importance to Canadian canola production and representative of the diversity in the *Brassica* sp. gene pool were seeded. Seeds were hand planted at a rate equivalent to 7 kg ha^{-1}. Counter 5-G (Cyanamid, USA) was applied in each row at 10 kg ha^{-1}, to prevent flea beetle damage. All seed were covered with soil which was lightly compacted. Prior to seeding, plots were fertilized with 50 kg ha^{-1} NH_4NO_3. The test areas were hand weeded.

There were three treatments, contol, +UV-B and ambient. There were four replications per treatment. UV-B 313 lamps were pre-aged for 100 hours to ensure a consistent UV-B irradiance. The UV-B lamps used were not perfect sources of UV-B but also supplied UV-A and UV-C radiation. In nature 99.9 % of the solar UV-C radiation is filtered-out by oxygen in the atmosphere. We filtered the UV-C from the UV-B treated plants. This produced three treatments:

1 **Ambient**: No overhead frame. Plot exposed to ambient solar radiation.

2 **+UV-B**: UV-B lamps wrapped with 0.076 mm thick cellulose acetate plastic. This supplied visible, **UV-A**, and **UV-B** radiation from the bulbs with ambient solar radiation.

3 **Control**: UV-B lamps wrapped with 0.15 mm thick mylar plastic. This treatment supplied visible and **UV-A** radiation from the bulbs with ambient solar radiation.

The cellulose acetate filters were changed every 7 days and mylar filters every 2 weeks, to prevent filter degradation. The UV-B irradiance from the lamp bank was measured between 280-320 nm at various distances beneath a UV-B frame with a spectrophotoradiometer (Optronics Model 752, Orlando, FL). A linear regression equation relating UV-B radiation to height above the plant canopy was used to maintain the correct UV-B dosage throughout the experiment. The UV-B lamps were turned on at 09:00 and off at 17:00 hrs on days without rain. When it rained the lamps were turned off to prevent equipment failure and greater UV-B exposure during period of low ambient solar irradiance. All treatments began after the seeds were planted and continued until the plants had matured and were harvested.

At maturity, 0.15 m from each plot end was trimmed and the remaining plants were harvested by hand counted and a representative 10 plant sub-sample removed from each plot for analysis. From each representative sample, pods were harvested, seeds threshed and weight and number determined. Yield per ha was established using the number of plants per plot and the 10 plant sub-sample yield.

Four times during the experiment the leaves were sampled to determine the effect of increased UV-B radiation on flavonoid pigment concentration. Leaf disks were removed from the leaves and the samples immediately frozen in liquid nitrogen and then stored at -70°C until flavonoids were extracted. Flavonoids were extracted by grinding the disk in a mortise with 10 ml of 70:1 methanol:hydrochloric acid (vol/vol) solution. The mixture was centrifuged to separate the plant organic debris from the flavonoids in the supernatant. A specrophotometer was used to measure the relative absorption of the solution at 325nm.

An analysis of variance was done on the yield, agronomic and flavonoid data. The most important comparison was the control versus the +UV-B treatment because in this treatment the plants received the same amounts of UV-A and UV-C radiation as well as the same degree of shading by the lamp frames.

Results:

1000 Seed Weight

An increase in UV-B radiation equivalent to a 20 % decrease in the ozone layer resulted in a generally a slight decrease in seed size for all cultivars except Bounty. Garrison and Hyola 401 had their seed size reduced the most by the increase in UV-B radiation. There was no real difference in the response to UV-B radiation between *B.napus* and *B. rapa* types.

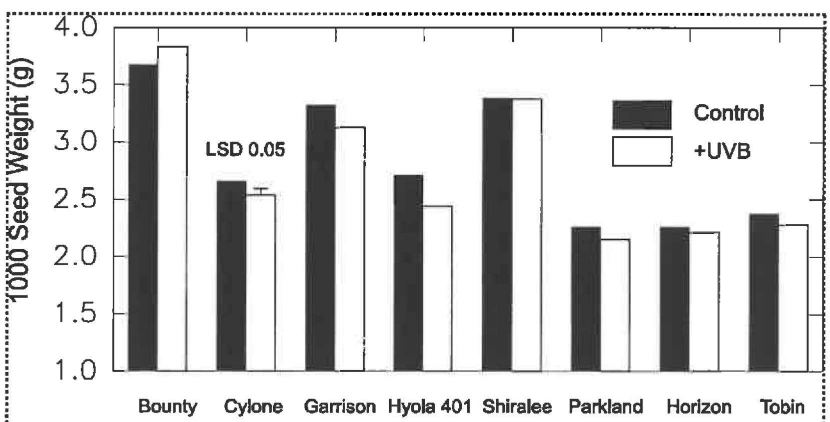

Fig. 1. 1000 Seed Weight of canola cultivars treated with UV-B radiation (+UV-B) equivalent to a 20% decrease in the ozone layer.

Flavonoid Pigment Concentration:

Flavonoid pigments absorb UV-B radiation. The pigments were measured at 320 nm using a specto-photometer. The greater the absorbance, the larger the amount of flavonoid pigment present. Increased UV-B radiation resulted in an increase in flavonoid pigment production in all cultivars. The response to UV-B among cultivars was variable. Some cultivars like Bounty, which had a high level of flavonoid pigment to begin with, produced less in response to UV-B while a cultivar like Garrison produced an abundance of flavonoid pigment in response to increase UV-B radiation. *B.napus* cultivars produced more flavonoid pigments initially and in response to UV-B then *B. rapa* cultivars.

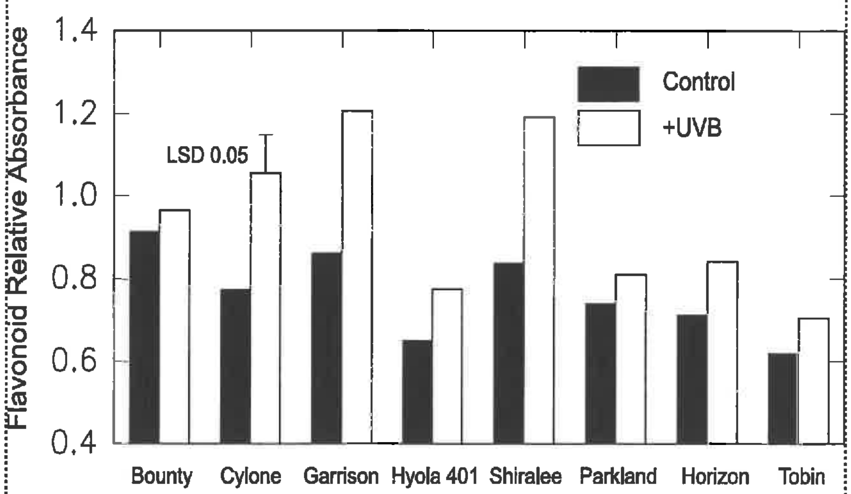

Fig. 2 Flavonoid pigment relative absorbance of canola cultivars treated with UV-B radiation (+UV-B) equivalent to a 20% decrease in the ozone layer.

Seed Yield

Seed yield was reduced with increased UV-B radiation in all of the B. napus lines and with 2 out of 3 B. rapa lines. There was variability for yield response to UV-B in both species, although it appears that yield was affected more in the *B. napus* than the *B. rapa* species. Under 1997 environmental conditions Cyclone and Bounty were affected the least by an increase in UV-B radiation equivalent to a 20 % decrease in the ozone layer. Hyola and Shiralee were

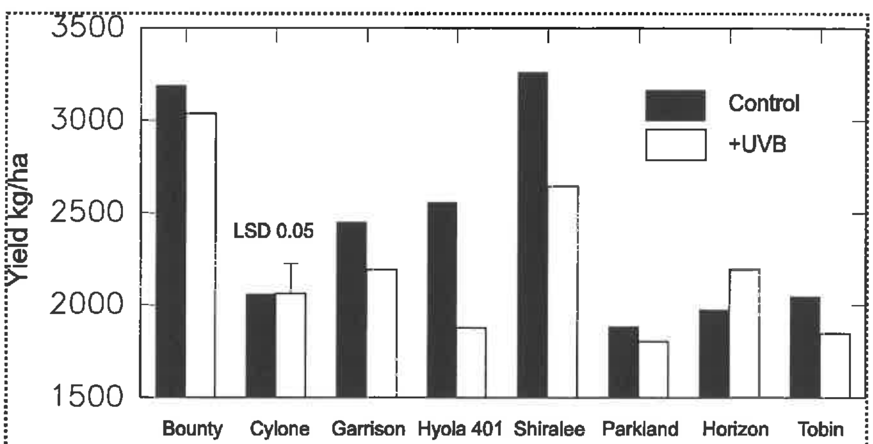

Fig 3. Yield of canola cultivars treated with UV-B radiation equivalent to a 20% reduction in the ozone layer.

affected the most by the increase in UV-B radiation. Within *B. rapa*, an increase in UV-B radiation resulted in an increase in the yield of Horizon, while AC Parkland and Tobin had yield reductions.

Table 1 shows that within both *Brassica napus* and *B. rapa* types of canola there is variability for tolerance to UV-B radiation. *B. napus* varieties seem more susceptible than *B. rapa* in 1997. The results are not consistent between years indicating that the environment interacts with UV-B radiation levels. Precipitation was higher in 1996 and temperatures lower. This is reflected in overall higher yields.

Table 1. Yield (kg/ha) of five *Brassica napus* and three *B. rapa* cultivars grown under enhanced UV-B radiation equivalent to a 20 % reduction in the ozone layer.

	1996			1997		
Variety	Control	+ UV-B	% diff*	Control	+ UV-B	% diff*
Bounty	2440	1765	28	3189	3036	5
Cyclone	3052	2255	26	2059	2061	0
Garrison	5546	6630	-20	2451	2191	11
Hyola 401	5978	6551	-4	2559	1876	27
Shiralee	3809	2141	44	3263	2645	19
Ave	4214	3868	15	2704	2362	12
AC Parkland	6450	3521	45	1885	1804	4
Horizon	7688	5591	27	1975	2191	-11
Tobin	3283	4275	-30	2047	1845	10
Ave	5807	4462	14	1969	1947	1

* %diff = yield reduction from the control ((control-UV-B)/control) x 100

Conclusions:

It is apparent, from the data, that some of the cultivars are tolerant to increased levels of UV-B but there is a strong interaction with the prevailing environmental conditions. There was no variety that appeared strongly resistant in both years, but Shiralee was clearly susceptible in both years. These results are positive proof that there is genetic variability for UV-B tolerance among canola cultivars. It seems that *B. rapa* cultivars are more tolerant to increases in UV-B radiation than *B. napus* cultivars. There is some consistency in the degree of average yield suppression in *B. napus* cultivars. In 1996 and 1997 there was a 15 and 12 % yield suppression with an increase in UV-B radiation equivalent to a 20 % decrease in the ozone layer over 1980 levels.

There does not seem to be a strong association between the amount of flavonoid pigment produced in response to UV-B and yield response. Screening for flavonoid pigment production may not be the best method to determine UV-B tolerance.

Even if all of the CFC reducing legislation (Montreal Protocol) is followed by all nations, the ozone layer is getting thinner each year and will continue to do so for at least the next 10 to 15 years. It will be well into the next century before the ozone layer is back to pre 1980 levels. This is a fact, not science fiction. This study has confirmed that canola is susceptible to UV-B radiation and yields are at risk.

Future Research

We must show that the results obtained in 1996 and 1997 are repeatable. Stress from UV-B radiation is much the same as stress from other environmental parameters. Often the variation among years is greater than the variation among lines within a year. We must begin to select today for the varieties of the near future. More work needs to be done to perfect a screening method to select for UV-B tolerant crops since flavonoid synthesis in response to increased UV-B radiation is not correlated with yield response to increased UV-B.