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The effects of increased UV-B radiation on growth, pollination success, and lifetime female fitness in two *Brassica* species

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Abstract The increasing levels of ultraviolet-B (UV-B) radiation reaching the earth's surface caused by ozone destruction have prompted many studies of UV-B effects on plants. Most of these studies have focused on physiological and growth responses of plants to increased UV-B, but these measures may not be closely related to future survival of plant populations. We examined the effects of two different levels of increased UV-B on total female fitness, including seed number and quality, in rapid-cycling strains of *Brassica nigra* and *B. rapa* (Brassicaceae). We also measured the effects of UV-B on fitness components, particularly those related to pollination success. Two separate experiments, examining two different levels of UV-B, were performed. Sixty plants of each species were grown under control and enhanced levels of UV-B for a total of 480 plants (60 plants×2 species×2 UV-B levels×2 experiments). Increased UV-B was generally detrimental to growth and flowering in both species; however, total seed production was actually greater at higher UV-B doses in three of four dose/plant species combinations examined. UV-B had little effect on pollination success or offspring quality in either species. Therefore, in spite of the detrimental effects of UV-B on growth and flowering that we found, there is little evidence that fitness of these plant species would suffer with increasing UV-B, and we caution against using solely physiological or growth measurements to infer effects of UV-B on plant population fitness.

Key words Ultraviolet radiation · Female fitness · *Brassica* · Pollination · Seed number and quality

Introduction

Destruction of stratospheric ozone has led to an increase in ultraviolet-B (UV-B) radiation reaching the earth. Recent studies estimate the rate of ozone loss in the Northern hemisphere as 2.3–6.7% per decade, resulting in an increase of 3–11% per decade in UV-B reaching the earth's surface (Blumthaler and Ambach 1990; van der Leun et al. 1991; Frederick 1993; Madronich 1993).

There have been numerous studies of the effect of UV-B on plants (see reviews by Teramura 1983; Caldwell et al. 1989; Krupa and Kickert 1989; Tevini and Teramura 1989; Bornman and Teramura 1993; Runeckles and Krupa 1994). Most of these studies have examined physiological and growth traits, and many have found reductions in these traits in response to increased UV-B. However, to predict how plant populations will respond to further ozone destruction, we must also understand the effects of increased UV-B on plant fitness. Virtually all of our knowledge of the effects of UV-B on plant fitness comes from studies of total mass of seeds or fruits produced in a few crop species for which seeds or fruits are the harvestable yield (see reviews by Teramura 1986; Krupa and Kickert 1989; Teramura 1990). Studies of UV-B effects on non-crop plant population fitness, defined as the number of seeds produced, are extremely rare, and have recently been designated as a research priority (Teramura 1990; SCOPE 1992).

In addition to measurements of seed number, a thorough understanding of UV-B effects on fitness requires knowledge of the factors and ecological processes determining seed number and seed quality. Pollination success is an important determinant of seed production in many species. The only studies of UV-B effects on pollination to date have examined in vitro pollen germination (e.g., Flint and Caldwell 1984 and references therein). Although these studies are important, in vitro germination may not correlate with in vivo germination (Mazer 1987; Cruzan 1990). In addition, increased UV-B may alter pollinator visitation, floral morphology, or the production of pollen, nectar, or flowers, all of which can reduce pollination success (e.g., Kay 1976; Stanton et al. 1986; Galen and Newport 1987; Thomson 1988; Campbell et al. 1991; Eckhart 1991; see review in Bertin 1988). The effects of UV-B on pollination is another area

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that has been recently designated as a research priority (SCOPE 1992).

UV-B can potentially affect seed quality in one of two ways. First, UV-B is a mutagen that may damage the embryo genetically, by causing mutations in the embryo itself or in the pollen and ovule that form the embryo (Caldwell et al. 1989). Second, UV-B may stress the maternal plant (e.g., through disruption of photosynthesis; see reviews cited above) and affect the quantity or quality of resources provided to the seed. The effects of UV-B on seed quality have received little empirical study (Caldwell et al. 1989; but see Teramura et al. 1990).

The goal of this study was to fill some of these gaps in our knowledge by measuring the effects of UV-B on four aspects of plant performance in two species of *Brassica*. First, we examined the effects of UV-B on growth and flowering. Second, we measured pollination success and plant traits that affect pollination, including floral morphology, pollen and nectar production, pollinator attraction, and pollen removal by pollinators. Third, we estimated total lifetime female fitness (seed production) and components of seed production. Finally, seed quality was estimated by measuring seed size, germination success, and offspring growth.

Materials and methods

Study organisms

Two species of Brassicaceae, *Brassica nigra* (black mustard) and *B. rapa* (syn. *campestris;* field mustard, turnip, rutabaga, oilseed rape, canola) were used for these experiments. This family is considered to be UV-B-sensitive based on studies of growth, photosynthesis, and leaf structure and composition (Van et al. 1976; Hashimoto and Tajima 1980; Tevini et al. 1981; Bornman and Vogelmann 1991; Cen and Bornman 1993; Wilson and Greenberg 1993). Brassicaceae are important crops worldwide (Kapil et al. 1971; Langridge and Goodman 1975), and oils and vegetables of *Brassica* species are essential crops in developing nations (Williams and Hill 1986). In addition, *Brassica* species are common agricultural weeds and colonists of disturbed habitats in the temperate zone. Both *B. nigra* and *B. rapa* are common weeds and are also raised as crops in some areas.

Experimental design

Rapid-cycling *Brassica* seeds were obtained from the Crucifer Genetics Cooperative (Madison, Wis.). These seed stocks were developed by combining natural populations with accessions from the USDA National Plant Germplasm System, and subjecting them to a number of generations of selection for early flowering time (Williams and Hill 1986; P. Williams, personal communication). Therefore, the plants we used are neither agricultural cultivars nor natural populations.

Two experiments, examining two different levels of increased UV-B, were performed. For each experiment, 120 plants of each species were grown in 8-cm pots using 1:1:1 peat:perlite:loam potting soil. Plants were watered daily, fertilized once a week with 20–20–20 fertilizer (236 PPm N), and grown in an unshaded, pollinator-free greenhouse. Half of the plants from each species (60 plants) were randomly assigned to one of two daily UV-B doses: 6 or 12 kilojoules (kJ m^{-2} $UV\text{-}B_{be300}$) in the first experiment (September 1993–October 1993) and 6 or 17 kJ in the second experiment (November 1993–December 1993). Therefore, a total of 480 plants were used in the two experiments: 60 plants×2 species×2 UV-B doses×2 experiments = 480. These dose measurements give the biologically effective daily UV-B dose weighted according to Caldwell's generalized plant damage spectrum (Caldwell 1971). The 6 kJ dose represents current levels of UV-B on a clear day on 1 June at 40° north latitude according to a standard model (Green et al. 1980) and serves as the control. Since these species grow and flower in the summer months, this is representative of average UV-B doses received by these plants in the field. The 12 and 17 kJ doses represent UV-B levels under the same conditions after approximately 30% and 45% destruction of the ozone layer, respectively.

UV-B was provided by one rack of 16 UV-B lamps (UV-B 313, Q-Panel Inc., Cleveland, Ohio) for each of two greenhouse benches. Before the experiment, lamps were illuminated for 100 h to stabilize UV-B output. Cellulose acetate filters (0.003" for 12 and 17 kJ and 0.005" for 6 kJ; thinner filters transmit more UV-B) were used to filter out UV-C wavelengths (<280 nm; Middleton and Teramura 1993). Filters were changed every 8–10 days because filter transmittance of UV-B decreases over time. Ultraviolet-B levels were recorded daily using a UV-B radiometer and datalogger system (SED 240, International Light, and LI 1000, Li-Cor; DeLucia et al. 1991) that was calibrated with a UV/VIS spectroradiometer (Optronics OL 752, Optronics Lab, Orlando, Fla.). Height of the light racks was adjusted daily to maintain proper UV-B doses, which were measured at the approximate median leaf height of the plants. UV-B was supplied 4 h daily centered around solar noon from before seeds germinated until after the plants senesced. Metal halide lamps (1000 W) supplied supplemental light of at least 212 μmol s^{-1} m^{-2} daily from 0600 to 2200 hours.

Since treatments were not replicated due to equipment limitations, steps were taken to ensure that the only systematic difference between the two treatment groups was UV-B. First, every 4 (early in the plants' lives) or 5 (late in the experiment) days the plants and UV-B treatments were switched to the opposite greenhouse benches. Therefore, treatments were interspersed spatially sensu Hurlbert (Hurlbert 1984). However, if the plants had sensitive developmental periods that occurred within the 4 or 5 days they spent on a single bench, and the benches differed systematically in some environmental variable other than UV-B, we would be unable to ascribe any differences between treatment groups unambiguously to UV-B effects.

To test for potential environmental differences between benches, we measured temperature and photosynthetically active radiation (PAR) at nine points on each bench. These measurements were taken at several times throughout the day on several different days with a variety of weather conditions throughout the experiments. There were no significant differences between the two benches in average temperature and PAR (average temperature 25.2 vs 25.3° C; average PAR 419 vs 413 μmol s^{-1} m^{-2}, Wilcoxon signed-rank tests, *P*-values ranging from 0.47 to 1.0). Systematic below-ground environmental differences between the treatments are unlikely, since all plants were planted in the same pots, using the same soil mix, and received identical water and fertilizer treatments.

Thus, major environmental differences between the treatment groups other than UV-B are unlikely, and our experimental unit is therefore individual plants. The error term for our ANOVA tests (see below) is therefore variance among plants within treatment groups; this variance is composed of genetic differences among plants and microenvironmental differences within treatment groups. To reduce the latter, plant positions were rotated within benches daily, and the two species were alternated within flats, with each flat containing six plants of each species.

Traits measured

Growth and flowering phenology

The following traits were measured weekly once plants developed their first true leaves: height from the soil surface to the tallest

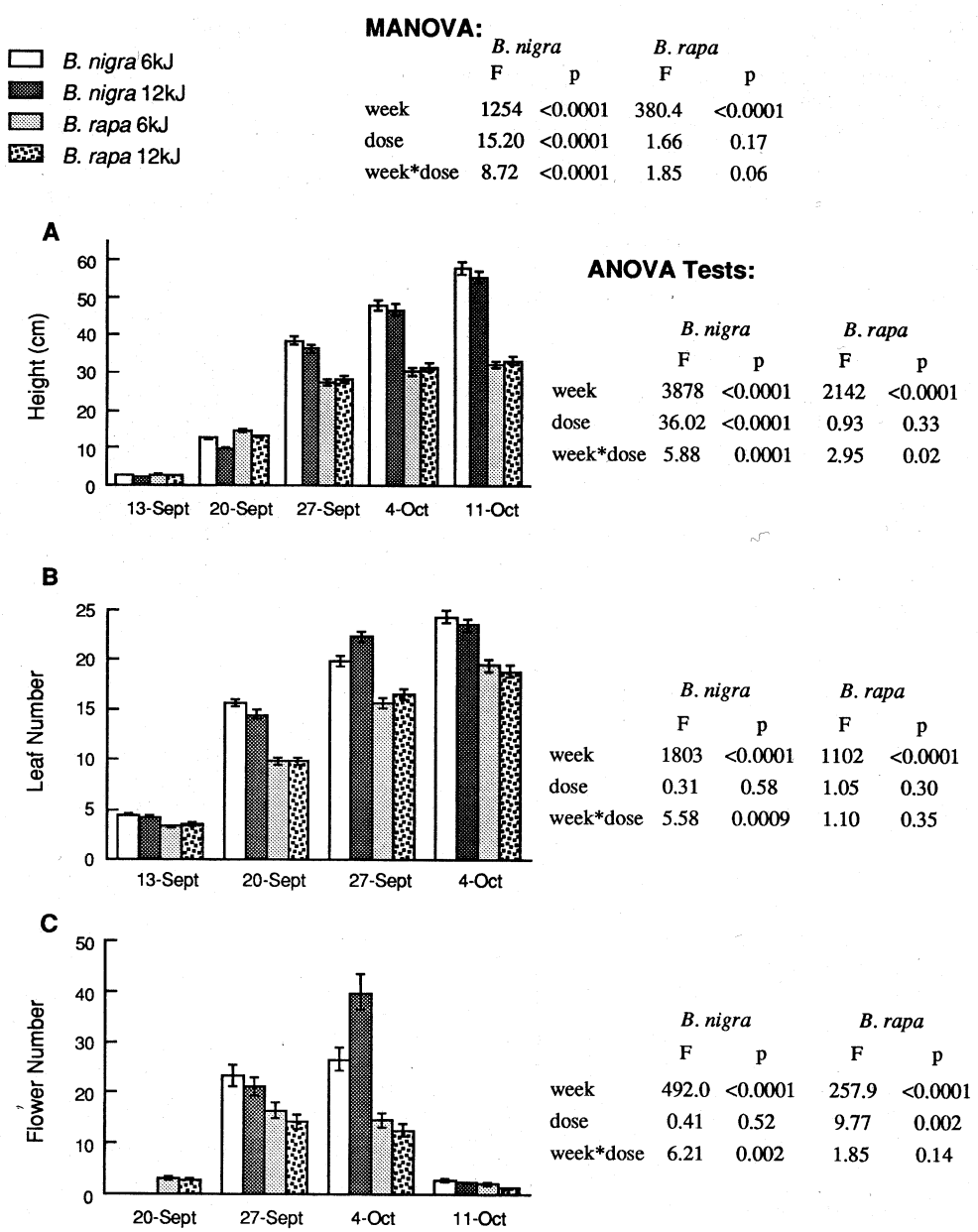

Fig. 1 Weekly growth and flower production results for the 6 versus 12 kJ experiment (Means±1 SEM). MANOVA table for all three traits simultaneously is at the top; ANOVA results for each trait are located to the right of their respective graphs. Total n=240

point of the plant, number of true leaves, and number of open flowers. Opening date of the first and last flower was also recorded for each plant.

Pollination and pollinator observations

Once plants started to flower, they were taken to an indoor 2.5 m×2.5 m×2.5 m flight cage containing a small honey bee hive for pollination. Pollination trials were completed before the UV-B lamps came on each day, so that plants always received their daily UV-B dose. Honey bees *(Apis mellifera)* were used as pollinators because they are important pollinators of *Brassica* species worldwide (Fries and Stark 1983). Thirty plants (15 of each species) grown under the same UV-B dose were interspersed in a 5×6 grid (1.2 m×1.5 m) on the cage floor. Exposing bees to plants grown at one UV-B dose at a time mimics a natural setting in which pollinators are often exposed to several species but never to plants grown at different UV-B doses. This arrangement also prevents fertilization of plants grown at one UV-B dose by pollen from a different dose.

After the first set of 30 plants was removed, 30 new plants from the same UV-B dose were brought into the cage for an equal amount of time (30–60 min). Thus, half the plants of one dose were pollinated at each session. The next time the same 60 plants were pollinated, the order in which the two sets of 30 were presented to the bees was reversed. These same plants were used concurrently in a separate honey bee pollination experiment. As a result, each plant was visited by bees 2 or 3 times weekly.

Pollinator observations were made by two observers on a pair of plants (one *B. nigra* and one *B. rapa*) with the observers alternating the species they observed. In this way, both species were equally represented at each observation session, and temporal variability and observer differences were controlled for across species. The height from the floor and the number of open flowers of focal plants were recorded before observations. Observations lasted 10 min per pair. The total number of bee visits was recorded, and the number of flowers probed per bee visit and the time spent per flower were recorded for a subset of visitors. These components multiply to equal total time spent by bees at the plant. A total of 73 and 101 pairs of plants were observed in

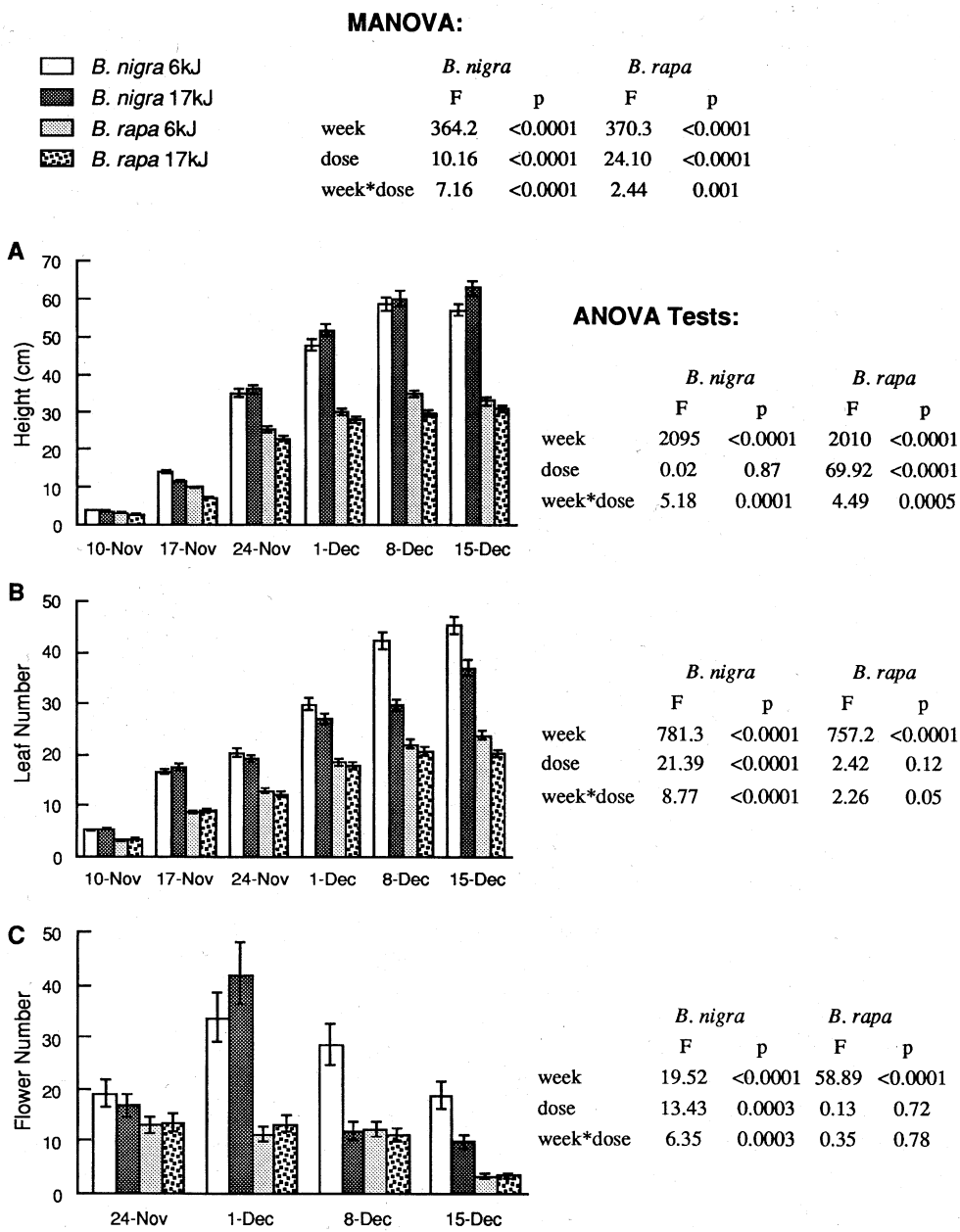

Fig. 2 Weekly growth and flower production results for the 6 versus 17 kJ experiment. See Fig. 1 legend for details. Total n=240

the first and second experiments, respectively; each plant was observed only once.

Pollen production and removal

Pollen collections were made before and after pollination sessions. Before pollination, a newly opened flower was removed and all six of its anthers were placed in a clean vial. Since this flower had opened in a pollinator-free greenhouse, this pollen sample represents pollen production before any removal by pollinators. Plants were then put in the flight cage as described above. After pollination, anthers of flowers adjacent to those gathered before pollination were collected. The difference in pollen counts between the flowers collected before and after pollination is an estimate of the number of pollen grains removed by the bees (Harder 1990; Young and Stanton 1990). This assumes that pollen production by adjacent flowers in similar. To test this, pollen counts were performed on two adjacent unvisited flowers from 15 plants. Results showed that 96% of variation in pollen counts occurred among plants. Thus, differences between "before and after" pollen counts were mainly due to pollen removal by bees.

After collection, uncapped pollen vials were put in a 37° C oven for a week to ensure complete anther dehiscence. Immediately before counting, vials were filled with approximately 20 ml of 2% NaCl and vortexed and sonicated repeatedly to remove pollen grains from anthers. Four counts of pollen grains present in 0.5 ml samples of solution were made with a Coulter counter. The four counts were averaged. Average counts were doubled (to give a per milliliter count) and then multiplied by the exact mass of NaCl solution added to give an estimate of the total pollen present in the vial.

Floral morphology

Six morphological measurements were made on a single flower from each of 70 plants in the 6 versus 12 kJ experiment (an average of 17.5 plants from each dose/species combination) and 80 plants

Table 1 Flowering phenology, fitness components, and floral morphology results for the 6 kJ versus 12 kJ experiment. In each group of variables, the first line presents the results of a MANOVA including all dependent variables in that group. If either the dose or dose*species tests in the MANOVA were significant at $P \leq 0.10$, then the results of individual ANOVAs for each dependent variable are presented separately

	Dose		Species		Dose*Species	
	F	*P*	*F*	*P*	*F*	*P*
MANOVA (flowering phenology)	5.62	0.004	209.76	<0.0001	2.10	0.13
Days to first flower	6.42	0.01	546.02	<0.0001	2.82	0.14
Flowering duration	5.42	0.02	1.72	0.19	0.00	0.95
MANOVA (fitness components)	1.78	0.09	153.60	<0.0001	0.97	0.45
Number of flowers	0.00	0.96	141.82	<0.0001	5.34	0.02
Number of fruits/flower	0.62	0.43	144.88	<0.0001	0.10	0.75
ln seeds/fruit	4.56	0.03	305.32	<0.0001	1.05	0.31
Number of seeds	8.64	0.003	1.05	0.31	0.20	0.65
Average mass/seed	2.58	0.11	112.99	<0.0001	0.21	0.65
Total above-ground biomass	1.27	0.26	4.90	0.03	0.24	0.62
Reproductive effort	5.39	0.02	134.93	<0.0001	0.74	0.40
MANOVA (floral morphology)	0.73	0.65	7.70	<0.0001	1.14	0.35

in the 6 versus 17 kJ experiment (exactly 20 plants from each dose/species combination). The following floral traits were measured (for details see Conner and Via 1993): the length and width of the distal, showy part of the petal (the "limb"), the length of the proximal part of the petal (the "claw", which forms a functional corolla tube in *B. rapa* but not *B. nigra*), the length of the pistil, and the lengths of one of the long filaments and one of the short filaments (Brassicaceae have four long and two short filaments).

Nectar (6 kJ vs 12 kJ only)

Another set of plants were grown under 6 and 12 kJ for a separate pollination experiment in the spring of 1994. These plants were used for nectar measurements. Nectar was collected from 10 flowers on each of 10 *B. rapa* plants from each dose using 1 µl microcapillary pipettes (*B. nigra* did not produce measurable amounts of nectar). Nectar volume was calculated from the length of the column of nectar in the pipette, measured with digital calipers. Sugar concentration of these samples was then measured using a pocket refractometer (Bellingham and Stanley, Tunbridge Wells, England). Collections were done in pairs (i.e. one plant from each UV-B dose simultaneously) to control for other environmental factors that might affect nectar production.

Fitness components

UV-B exposure continued until plants senesced. Plants were then harvested and the total number of flowers (pedicels), fruits, and seeds produced by each plant were counted. Air-dried fruits, seeds, and the rest of the above-ground plant were weighed separately. Thirty-two seeds were then randomly sampled from each plant's seeds for offspring experiments. From these data, we calculated three fitness components: number of flowers, number of fruits/flower (= % fruit set), and number of seeds/fruit (calculated as the total number of seeds divided by the total number of fruit produced); these multiply to equal total lifetime seed production. We also calculated total above-ground biomass (fruit mass + stem mass) and reproductive effort (fruit mass/total above-ground biomass).

Offspring measurements

To study the potential effects of UV-B exposure on offspring quality, the 32 seeds that were randomly sampled from each experimental plant (see above) were planted in two 8-cm pots (16 seeds/pot) in MetroMix 360 potting soil for a total of 15 360 seeds sown in both experiments (32 seeds×480 plants). These offspring were grown in a pollinator-free greenhouse without UV-B. This enabled us to isolate the effects of UV-B on seed quality without confounding this with direct effects of UV-B on the offspring plants. Differences between UV-B treatments in offspring traits could be due either to direct effects on the seeds that produced those offspring, or differences between UV-B treatments in selection among plant genotypes creating genetic differentiation between the germinating offspring of the two treatment groups. Our experiment could not distinguish between these possibilities. To control for microenvironmental effects, seeds from different UV-B dose treatments and species were interspersed within flats.

After recording the total number of seeds germinating, seedlings were randomly thinned to one per pot. The number of true leaves and height (from the soil to the tallest ponint of the plant) were recorded either 2 weeks *(B. rapa)* or 3 weeks *(B. nigra)* after planting. These measurements were again taken on the day the plants first flowered (height was taken from the soil to the highest open flower). Since there were two offspring per parent, averages were taken for each parental plant. In addition, floral morphology of offspring was measured (as above) in the 6 kJ versus 17 kJ experiment. One offspring from each of 120 parents was measured (30 of each species at each dose).

Analyses

Because we measured a large number of traits, there are a very large number of possible statistical tests. Our approach to this problem throughout was to group related variables together and perform multivariate analyses of variance (MANOVA) to test for differences caused by UV-B for the group of traits as a whole. If a multivariate test was significant at $P \leq 0.10$, univariate tests were conducted on each variable separately to determine which was affected most by UV-B. All independent variables were considered to be fixed effects, so all *F*-tests used the error Mean Square in the denominator. Some dependent variables were ln-transformed to reduce heteroscedasticity (increasing variance with increasing magnitude of the trait; see Tables 1, 2, 4). Little or no heteroscedasticity remained after transformation, as indicated by visual inspection of residual plots. All statistical tests were performed using JMP on Macintosh computers (SAS Institute 1994).

Weekly measurements

MANOVA was used to test for effects of week, dose, and the week by dose interaction on the three traits measured together (height, leaf, and flower number).

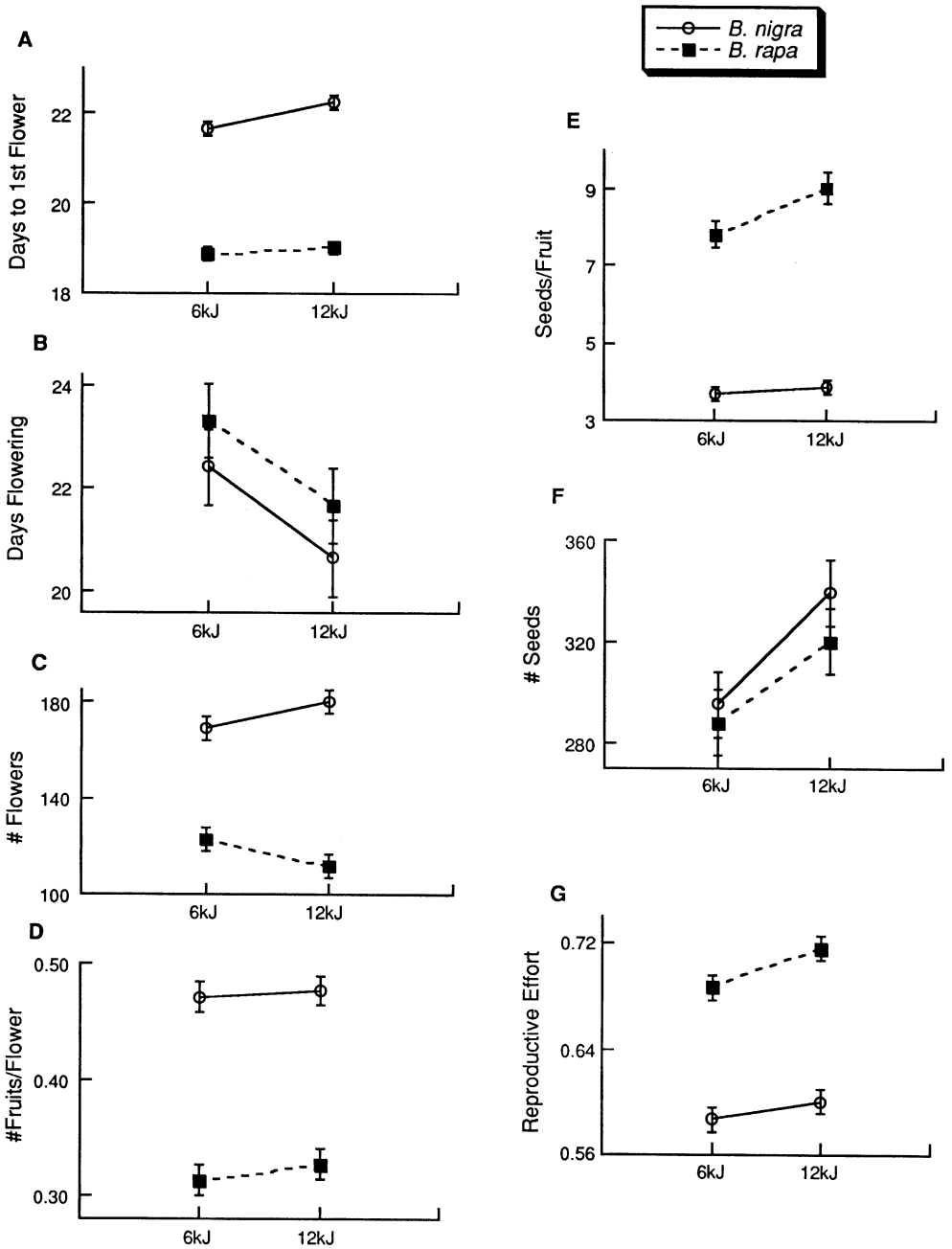

Fig. 3 Means and ±1 SEM for flowering phenology and fitness components in the 6 versus 12 kJ experiment. Only traits for which dose or dose*species were significant at $P \leq 0.05$ (Table 1) are presented, except that all fitness components (number of flowers, number of fruits/flower, number of seeds/fruit, and number of seeds) are presented for completeness. ln(seeds/fruit); (Fig. 3E) was back transformed for ease of interpretation. Reproductive effort was measured as (Dry fruit mass)/(Total plant dry mass). n=229 for days flowered, n=239 for all other traits

Single measurements

Traits that were measured one time on all plants were split into five categories: flowering phenology (number of days to first flower, flowering duration), fitness components (number of flowers, number of fruits/flower, number of seeds/fruit, number of seeds, average mass/seed, total above-ground biomass, reproductive effort), floral morphology (petal limb length and width, petal claw length, pistil length, long filament length, and short filament length), offspring growth (percent germination, first and second measurements of height and leaf number, and number of days until first flower), and offspring floral morphology (same traits as above). MANOVAs were run for each category separately with dose, species, and dose*species as independent variables.

Pollinator visitation

Pollination data were split into two categories: plant traits (height and flower number) and visitation by pollinators (number of visits, number of flowers probed/bee visit, and time spent/flower).

Pollen production/removal

ANOVA was used to determine effects of species, dose and species*dose on pollen production and pollen removal by honey bees.

Results

Weekly growth measurements

Figure 1 and 2 show weekly morphological measurements along with ANOVA and MANOVA results. In all cases, there was a strong week main effect, which indi-

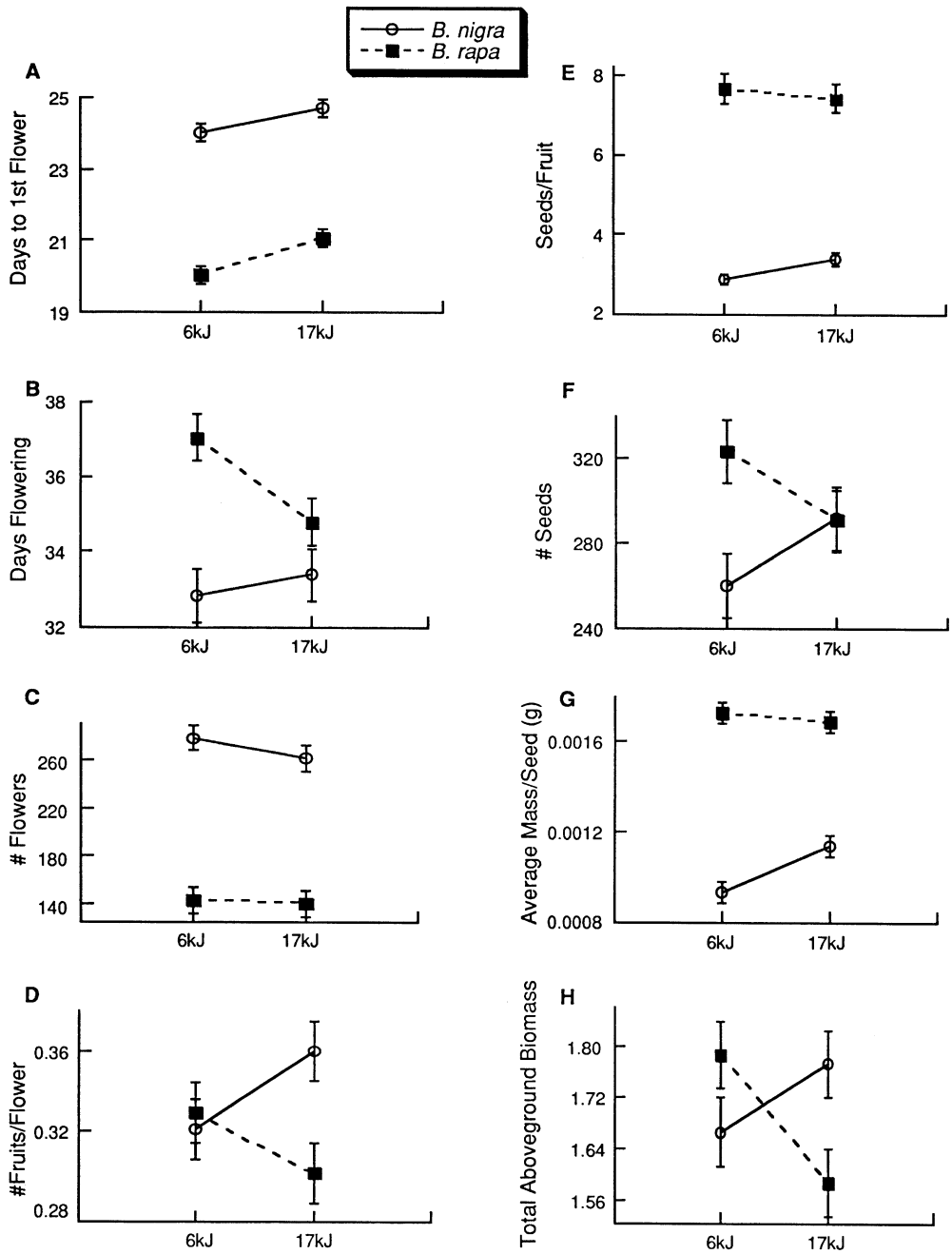

Fig. 4 Means and ±1 SEM for flowering phenology and fitness components in the 6 kJ versus 17 kJ experiment. See Fig. 3 legend for details. n=196 for days flowered, n=238 for all other traits

cates only that plants were growing over time. In the 6 versus 12 kJ experiment (Fig. 1), UV-B had a much stronger effect on *B. nigra* than *B. rapa:* the MANOVA showed a highly significant dose main effect and week*dose interaction in *B. nigra,* but no effect of dose and a marginally significant week*dose interaction in *B. rapa.*

Increased UV-B caused significant reductions in height in *B. nigra* and flower number in *B. rapa* (Fig. 1, significant dose main effects). In both species increased UV-B caused a reduction in height in the 2nd week, but this effect lessened in subsequent weeks (Fig. 1, significant week*dose interaction). *B. nigra* also showed a reduction in leaf and flower number with increased UV-B early in life, but this later reversed (Fig. 1, significant week*dose interaction). This result, combined with the lack of a significant UV-B dose main effect, suggests that increased UV-B caused a delay in leaf and flower production in *B. nigra,* but no overall reduction in either trait (see flowering phenology and lifetime flower production results below).

Plants responded more dramatically to UV-B in the 6 versus 17 kJ experiment (Fig. 2). MANOVAs showed highly significant dose main effect and week*dose interaction in both species.

Increased UV-B caused reductions in height of *B. rapa* and leaf and flower number in *B. nigra* (significant dose main effects, Fig. 2A–C). Significant week*dose interactions occurred in every trait except flower number in *B. rapa.* In *B. nigra,* increased UV-B caused plants to

Table 2 Flowering phenology, fitness components, and floral morphology results for the 6 kJ versus 17 kJ experiment. See Table 1 for details

	Dose		Species		Dose*Species	
	F	*P*	*F*	*P*	*F*	*P*
MANOVA (flowering phenology)	5.55	0.004	101.32	<0.0001	2.24	0.11
Days to first flower	11.35	0.0009	229.95	<0.0001	0.45	0.50
Flowering duration	1.70	0.19	17.65	<0.0001	4.50	0.04
MANOVA (fitness components)	0.94	0.47	92.72	<0.0001	4.16	0.0002
Number of flowers	0.88	0.35	140.26	<0.0001	0.49	0.49
Number of fruits/flower	0.09	0.76	2.91	0.09	5.11	0.02
ln seeds/fruit	1.72	0.19	305.15	<0.0001	3.84	0.05
Number of seeds	0.00	0.98	4.24	0.04	4.65	0.03
Average mass/seed	3.05	0.08	209.53	<0.0001	7.05	0.008
Total above-ground biomass	0.75	0.39	0.37	0.54	8.30	0.004
Reproductive effort	3.02	0.08	142.82	<0.0001	2.64	0.11
MANOVA (floral morphology)	1.38	0.23	33.88	<0.0001	0.52	0.82

Table 3 Effects of UV dose and species on pollinator attraction. See Table 1 for details

	Dose		Species		Dose*species	
	F	*P*	*F*	*P*	*F*	*P*
6 versus 12 kJ						
MANOVA (Plant traits)	2.75	0.07	32.24	<0.0001	1.12	0.33
Height	0.00	0.95	58.70	<0.0001	0.17	0.68
Number of flowers	5.65	0.02	17.78	<0.0001	2.45	0.12
MANOVA (Pollinator visitation)	3.25	0.02	2.70	0.05	0.29	0.83
Number of visits	0.33	0.57	0.10	0.75	0.63	0.43
Flowers probed/Bee visit	2.81	0.10	4.44	0.04	0.00	0.98
Time/Flower	4.48	0.04	5.10	0.03	0.52	0.47
6 versus 17 kJ						
MANOVA (Plant traits)	6.57	0.002	61.62	<0.0001	7.55	0.0007
Height	9.46	0.002	121.01	<0.0001	15.10	0.0001
Number of flowers	8.36	0.004	29.48	<0.0001	1.22	0.27
MANOVA (Pollinator visitation)	6.42	0.0004	1.53	0.21	0.88	0.45
Number of visits	0.84	0.36	0.05	0.83	0.02	0.90
Flowers probed/Bee visit	13.49	0.0003	1.15	0.28	1.84	0.18
Time/Flower	4.17	0.04	3.22	0.07	0.62	0.43

be shorter when young but taller when older (Fig. 2A). *B. rapa* plants exposed to 17 kJ were shorter throughout the experiment, but the difference in heights between doses varied from week to week (Fig. 2A). Younger *B. nigra* plants had similar numbers of leaves and flowers under both UV-B doses, whereas older plants had fewer leaves and flowers under increased UV-B (Fig. 2B, C).

Flowering phenology and fitness components

The MANOVA results for the 6 versus 12 kJ experiment showed that UV-B had a significant effect on flowering phenology and a marginally significant effect on the fitness components (Table 1, dose main effects). No other significant effects of UV-B were found except a marginally significant dose*species interaction for flowering phenology.

The 12 kJ dose significantly increased days to first flower while significantly decreasing flowering duration in both species (Fig. 3A, B, Table 1). In spite of this potentially detrimental effect, both species showed significant increases in seed production as the UV-B dose increased from 6 to 12 kJ (Fig. 3F; Table 1 significant dose main effect but no dose*species interaction). This increase was achieved through different fitness components in the two species. Increased seed production in *B. nigra* at 12 kJ was due mainly to increased number of flowers produced. Decreased flower production in *B. rapa* was offset by higher numbers of seeds per fruit at the higher UV-B dose (Fig. 3C–E). Fruit set was not altered by UV-B in either species. The increase in total number of flowers produced by *B. nigra* at 12 kJ was in spite of the decreased flowering duration, indicating that plants had a higher rate of flower production at higher UV-B levels. Reproductive effort was significantly greater at 12 kJ in *B. rapa,* and possibly in *B. nigra* as well (Fig. 3G, Table 1).

The MANOVA results for the 6 versus 17 kJ experiment show that UV-B had a highly significant effect on

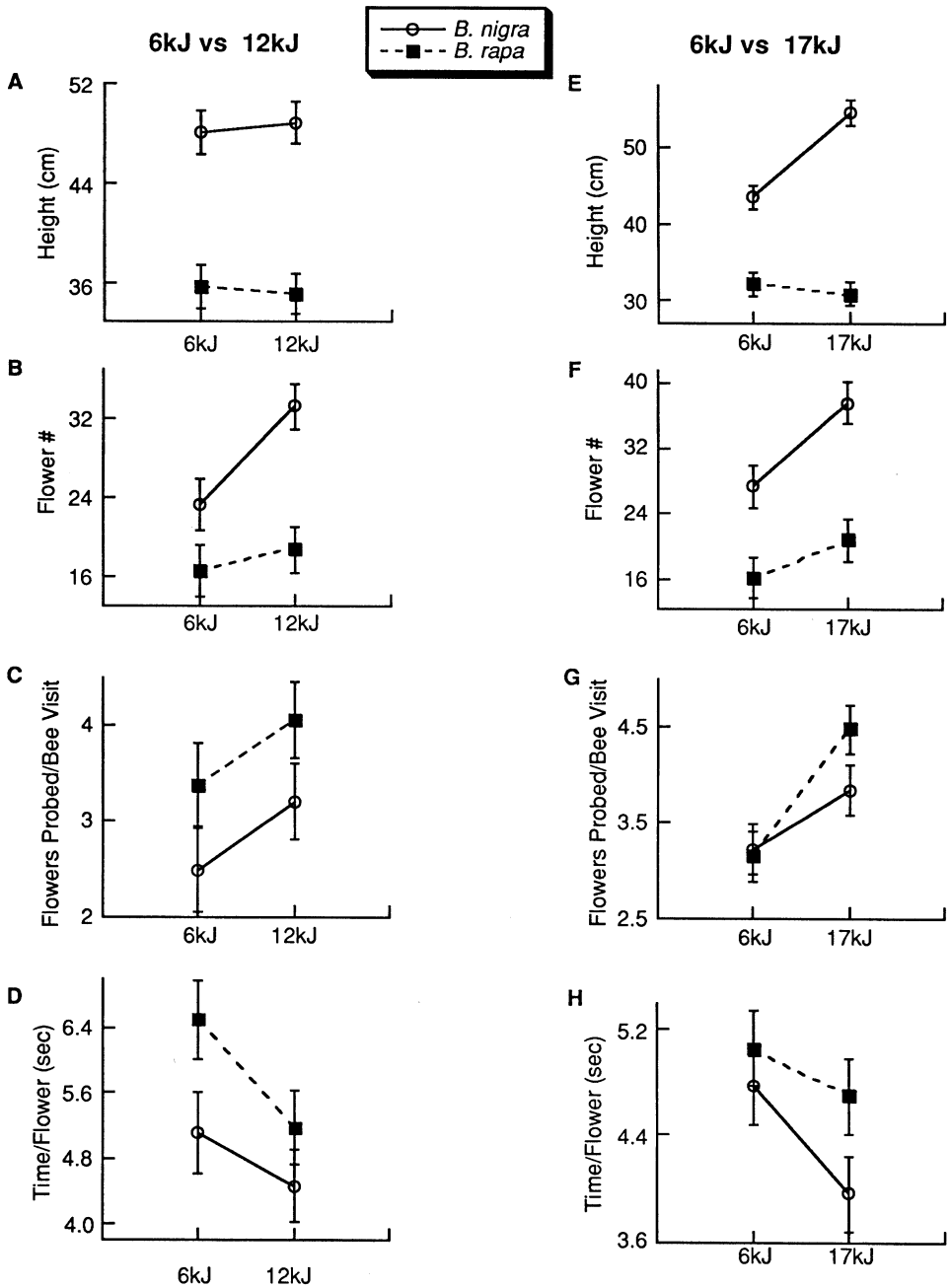

Fig. 5 Means and ±1 SEM for pollinator visitation trials. Only traits for which dose or dose*species were significant at $P \leq 0.05$ (Table 3) are presented. For the 6 versus 12 kJ experiment, $n=146$; for the 6 versus 17 kJ experiment, $n=202$

flowering phenology (Table 2, dose main effects). There also was a highly significant dose*species interaction for the fitness components.

Plants of both species exposed to 17 kJ UV-B took longer to flower (Fig. 4A, Table 2); this result was consistent with the longer time to flower for both species at the higher dose in the 6 versus 12 kJ experiment. Duration of flowering in *B. rapa* decreased with increasing UV-B as in the 6 versus 12 kJ experiment. Increased UV-B did not significantly affect flowering dura-tion in *B. nigra,* in contrast to the 6 versus 12 kJ experiment (Fig. 4B, Table 2, significant dose*species interaction).

B. nigra showed an increase in seed production with increased UV-B, as in the 6 versus 12 kJ experiment, but *B. rapa* showed a decrease (Fig. 4F, Table 2, no significant dose main effect but a significant dose*species interaction). This pattern of seed production was due mainly to differences in fruits/flower and, to a lesser degree, seeds/fruit (Fig. 4A, E, Table 2). Similar dose*species interactions occurred for average mass/seed and total above/ground biomass (Fig. 4G, H, Table 2), with *B. nigra* increasing and *B. rapa* decreasing at the higher UV-B dose.

Pollination success

UV-B did not affect nectar production (6 kJ: 0.59±0.11 µl; 12 kJ: 0.59±0.11 µl; means±SD, paired $t=0.03$,

Table 4 Offspring growth measurements for both experiments and offspring floral morphology for the 6 versus 17 kJ experiment. See Table 1 for details. Leaf number 1, leaf number 2, height 1, and height 2 refer to the first and second measurements of leaf number and overall plant height, respectively

	Dose		Species		Dose*Species	
	F	*P*	*F*	*P*	*F*	*P*
6 versus 12 kJ						
MANOVA (growth)	1.01	0.42	690.01	<0.0001	1.80	0.10
% Germination	0.08	0.78	16.05	0.0001	3.85	0.05
ln Leaf number 1	0.58	0.45	569.07	<0.0001	0.07	0.79
Height 1	1.93	0.17	143.31	<0.0001	0.83	0.36
ln Leaf number 2	0.05	0.82	457.22	<0.0001	1.61	0.21
Height 2	0.10	0.76	2924.70	<0.0001	5.32	0.02
Days To First Flower	2.74	0.10	603.07	<0.0001	0.78	0.38
6 versus 17 kJ						
MANOVA (growth)	2.01	0.07	964.96	<0.0001	1.00	0.42
% Germination	1.62	0.20	122.13	<0.0001	1.25	0.27
ln Leaf number 1	2.15	0.14	1578.00	<0.0001	1.20	0.28
Height 1	0.10	0.75	341.68	<0.0001	0.01	0.91
ln Leaf number 2	0.00	0.97	1272.60	<0.0001	0.18	0.67
Height 2	5.54	0.02	159.71	<0.0001	0.70	0.40
Days To First Flower	1.36	0.24	873.43	<0.0001	4.74	0.03
6 versus 17 kJ						
MANOVA (floral morphology)	1.23	0.30	24.79	<0.0001	2.33	0.04
Petal limb length	0.68	0.41	0.18	0.68	6.62	0.01
Petal limb width	3.93	0.05	0.57	0.45	1.50	0.22
Petal claw length	0.58	0.45	39.07	<0.0001	0.09	0.76
Pistil length	0.60	0.44	4.65	0.03	7.23	0.008
Long filament length	0.27	0.60	2.97	0.09	0.91	0.34
Short filament length	0.16	0.69	21.14	<0.0001	0.64	0.42

P=0.98) or concentration (6 kJ: 71.8±1.2%; 12 kJ: 71.9±1.4%; means±SD, paired t=0.17, P=0.87) in *B. rapa*. UV-B also did not significantly affect pollen production by the plants or pollen removal by honey bees in either experiment (data not shown). In each experiment *B. rapa* produced more pollen than *B. nigra*.

In both the 6 versus 12 and 6 versus 17 kJ pollinator attraction experiments, both the number of flowers open on the plants and the number of flowers probed per visit by bees increased with increased UV-B dose, but the time spent per flower decreased (Table 3, Fig. 5). In contrast, UV-B had no effect on the total number of bees visiting each plant in either experiment. In the 6 versus 12 kJ experiment only, bees probed more flowers per visit and spent more time per flower on *B. rapa* than *B. nigra* in spite of the fact that *B. nigra* had more flowers open during observations. This result is probably due to the greater per flower pollen and nectar production by *B. rapa* (see above).

To summarize the visitation results, increased UV-B caused no detrimental changes in pollination across UV-B doses. In each experiment, bees probed more flowers on plants of the higher UV-B dose; however, this was offset in both experiments as bees spent less time per flower on 12 and 17 kJ plants (Fig. 5). There were no species*dose interactions in visitation by pollinators in either experiment (Table 3). Thus, we would expect no interspecific shifts in competition for honey bee pollinators with increased UV-B.

Offspring quality

Parental UV-B levels did not have a strong effect on offspring traits in either experiment. The MANOVA for offspring growth in the 6 versus 12 kJ experiment showed no dose main effect and only a marginally significant dose*species interaction (Table 4). Two of the individual traits exhibited this interaction: both percent germination and the second height measurement increased in *B. nigra* and decreased in *B. rapa* at the higher dose (Table 4, Fig. 6A, B).

The MANOVA for the 6 versus 17 kJ experiment showed a marginally significant effect of UV-B on offspring growth, and a weakly significant dose*species interaction for offspring floral morphology. Offspring of parents exposed to 17 kJ were taller at the second measurement in both species (Fig. 6C). In addition, there was a significant dose*species interaction in average days to first flower with *B. rapa* taking longer to flower when their parents were treated with 17 kJ compared to offspring of the 6 kJ plants (Fig. 6D).

For offspring floral morphology, there were significant dose*species interactions for petal limb and pistil length, and a significant dose main effect for petal limb width (Table 2). *B. rapa* plants exposed to 17 kJ produced offspring with shorter and narrower petals (Fig. 6E, F) and shorter pistils (Fig. 6G) relative to offspring produced at 6 kJ. Conversely, *B. nigra* plants exposed to 17 kJ produced offspring with longer petals and

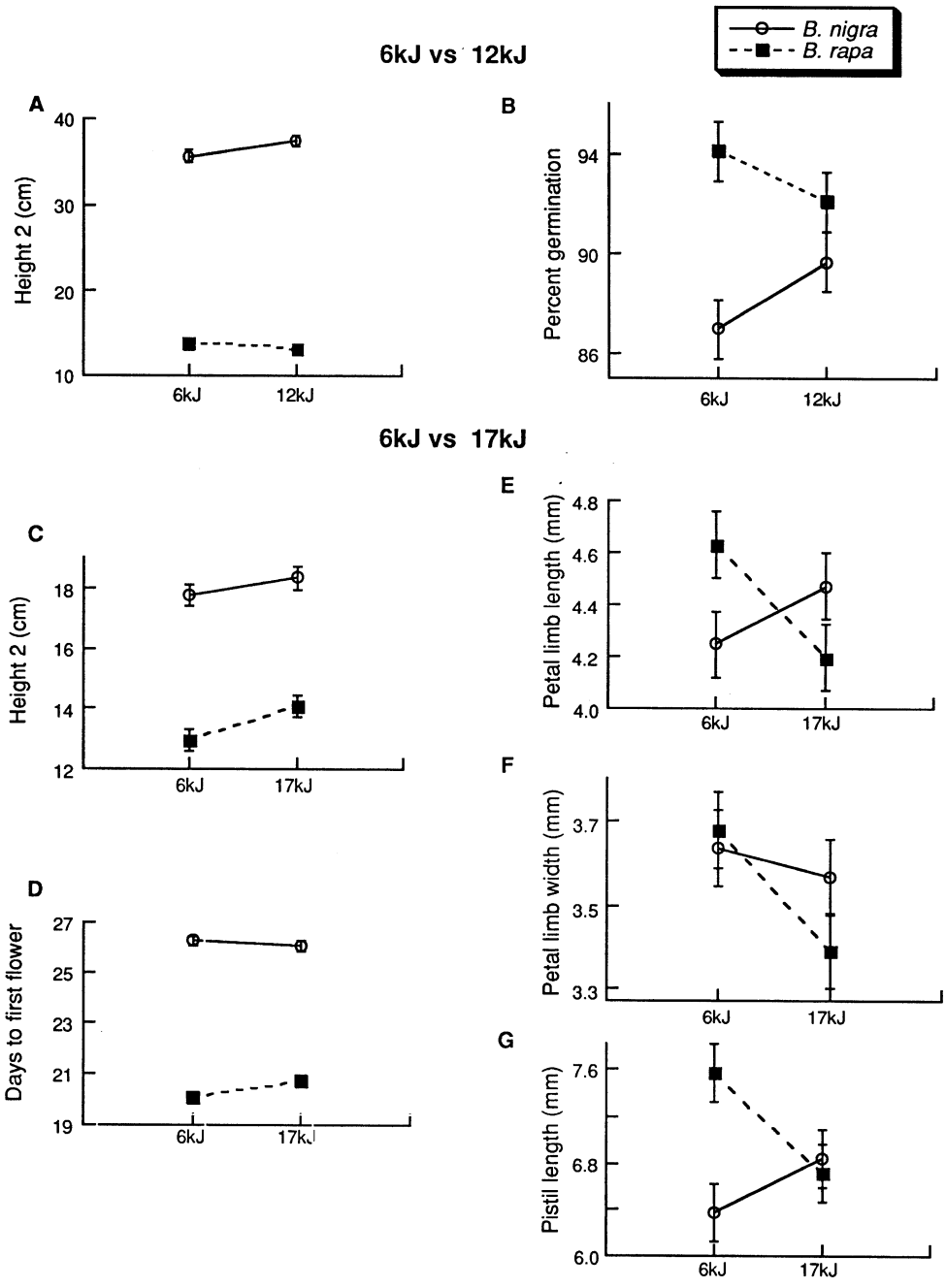

Fig. 6 Offspring results from both experiments. See Fig. 3 for details. ln(Height 2) has been back transformed for easier interpretation. n=476 for offspring traits, n=120 for offspring floral morphology

pistils (Fig. 6E, G) than those produced at the lower dose.

Discussion

Our results (summarized in Table 5) indicate that fitness of *Brassica* populations may not be reduced in the short-term as ambient UV-B levels increase. Lifetime seed production increased with increased UV-B in every case except one (*B. rapa* 6 vs 17 kJ), and there was little overall change in offspring quality. Most of the other studies that have measured the effects of UV-B on plant fitness or fitness components have examined crop plants, and few studies have actually counted numbers of seeds (see below). Several studies have measured total mass of all seeds (which includes seed number and seed size), and many more have measured total mass of all fruits (reviewed by Teramura 1986, 1990; Krupa and Kickert 1989); both of these traits are likely to be related to seed number, especially the former.

In a 6-year field study of two cultivars of soybean at two different enhanced UV-B doses (Teramura et al. 1990), total seed number increased significantly in seven experiments, decreased in five experiments, and did not change significantly in ten experiments. Of 11 studies that measured total mass of seeds in five different seed crops (rice, wheat, corn, soybean, and peanut), 4 report-

Table 5 Summary of increased UV-B effects on *Brassica nigra* and *B. rapa* for both experiments

Probably detrimental:

Weekly growth measurements
- ↓height *B. nigra*-12 kJ
- ↓height *B. rapa*-17 kJ
- ↓leaf number *B. nigra*-17 kJ
- ↓flower number *B. rapa*-12 kJ
- ↓flower number *B. nigra*-17 kJ

Flowering Phenology
- ↑time to first flower *B. nigra*-12 kJ
- ↑time to first flower *B. nigra*-17 kJ
- ↑time to first flower *B. rapa*-17 kJ
- ↓flowering duration *B. nigra*-12 kJ
- ↓flowering duration *B. rapa*-12 kJ
- ↓flowering duration *B. rapa*-17 kJ

Fitness and fitness components
- ↓number of flowers *B. rapa*-12 kJ
- ↓number of seeds *B. rapa*-17 kJ
- ↓above-ground biomass *B. rapa*-17 kJ

Offspring
- ↑time to first flower *B. rapa*-17 kJ
- ↓petal limb size *B. rapa*-17 kJ
- ↓pistil length *B. rapa*-17 kJ

Probably beneficial:

Fitness and fitness components
- ↑number of flowers *B. nigra*-12 kJ
- ↑number of fruits/flower *B. nigra*-17 kJ
- ↑number of seeds/fruit *B. rapa*-12 kJ
- ↑number of seeds/fruit *B. nigra*-17 kJ
- ↑number of seeds *B. nigra*-12 kJ
- ↑number of seeds *B. rapa*-12 kJ
- ↑number of seeds *B. nigra*-17 kJ
- ↑average mass/seed *B. nigra*-17 kJ
- ↑above-ground biomass *B. nigra*-17 kJ
- ↑reproductive effort *B. rapa*-12 kJ

Offspring
- ↑% germination *B. nigra*-12 kJ
- ↑average height 2 *B. rapa*-17 kJ

Little or no effect:
- Pollen produced
- Pollen removed
- Pollinator visitation rates
- Nectar volume and concentration
- Floral morphology

ed that increased UV-B decreased total seed mass and 7 reported no significant change (Teramura 1986, 1990). Many other studies have examined the effects of increased UV-B at a variety of dose levels on total fruit mass produced by crop plants (reviewed by Teramura 1986, 1990; Krupa and Kickert 1989). These studies have generally found no change or decreases in fruit mass with increased UV-B, but a few have found increases. Therefore, our finding of significant increases in lifetime seed production in three of four cases in unusual, although one of the most extensive of the studies reviewed above did find increases in total seed number in a substantial number of cases (Teramura et al. 1990).

There are at least two possible reasons for these differences between our fitness results and those of the previous studies. First, we did not use agricultural cultivars; it is possible that selective breeding of crop cultivars often reduces a plant's ability to deal with UV-B stress. The plants we used, however, were derived from agricultural plants and subjected to intensive selective breeding for rapid development. In one study of a non-crop plant that is in the family Brassicaceae, increased UV-B had no effect on the number of fruits per plant in *Arabidopsis thaliana* (Usmanov et al. 1987). More studies of fitness effects on non-crop species are needed, especially plants from natural populations.

Second, our studies were conducted in the greenhouse where the plants were provided with ample visible light, water, and nutrients. It may be that under these ideal conditions, plants are able to increase their seed output in response to UV-B, but that under more stressful natural conditions seed outputs would decline. This explanation is not supported by previous studies, however. Some of the studies cited above were also in the greenhouse, and the one study that showed increased seed production with increased UV-B was conducted in the field (Teramura et al. 1990). In addition, some earlier work combining water and nutrient stresses with UV-B, mainly in soybeans, have found that detrimental effects of UV-B tend to be masked by other stresses (reviewed in Bornman and Teramura 1993). Additional studies that examine the effects of other stresses in combination with UV-B would be very illuminating, especially in plants other than soybean (Teramura 1990).

In addition to total seed number, the quality of the seeds is crucial to plant fitness. We found no major detrimental effects of UV-B on seed quality, measured as seed size, germination percentage, and growth and flowering of the resulting plants. Thus, there was no evidence for UV-B induced mutations in our experiments. Our study lasted only one generation, however; mutations may accumulate over many generations of UV-B exposure. Studies that measure the effects of multiple generations of UV-B exposure are necessary to fully explore the effects of UV-B induced mutations on plant traits and population fitness. Also, in spite of some evidence for plant stress caused by UV-B (reduced growth and flowering phenology changes), plants did not reduce allocation to seed endosperm, as reflected in seed mass, germination, and seedling growth. Little is known about UV-B effects on seed quality in plants. A notable exception to this is the 6-year field study of soybean (Teramura et al. 1990), which found some small increases and decreases in protein and oil content of seeds in response to increased UV-B, but overall little change in seed quality.

In contrast to our results for fitness, we found detrimental effects of UV-B on a variety of growth and flowering phenology traits (Table 5). Thus, we caution against inferring effects of UV-B on fitness based solely on growth responses. For example, leaf number in *B. nigra* was significantly reduced at 17 kJ, while seed num-

ber significantly increased compared to the 6 kJ dose. However, it is possible that if the plants were growing under more competitive conditions in the field, the reductions in leaf number might have resulted in reductions in seed production (cf. the above discussion about the effects of stresses other than UV-B).

Even results based on reproductive traits might not indicate effects on total fitness. For example, *B. rapa* exposed to 12 kJ showed a significant decrease in flower number but a significant increase in total seed number compared to the control dose due to increased numbers of seeds/fruit. Therefore, studies that show a decrease in flower production with increased UV-B (reviewed in Tevini and Teramura 1989) may not necessarily indicate detrimental effects on fitness.

We found no detrimental effects of UV-B on pollination success in either experiment. Floral traits such as petal size, pollen and nectar production, which are known to affect pollination success, did not decline, and no reduction in pollinator attraction was observed. A companion study also found no effect of UV-B exposure on honey bee foraging behavior on *Brassica* (Collins et al., submitted), although neither this study nor ours examined direct effects of UV-B on bees. Our experiments provided consistently high levels of pollinators, however; field conditions in which pollinators may be limited and intra- and inter-specific competition for pollinators is occurring might yield different results. Higher levels of UV-B did increase the days until flowering and decrease total flowering duration in most cases, but this is unlikely to greatly affect pollinator visitation rates in the field for two reasons. First, the differences in flowering phenology were only 1 or 2 days. Second, these species flower in mid-summer and are visited by a variety of generalist pollinators. Timing of flowering could be much more important in species that flower early in the spring or that rely on specialist pollinators. Since this is the first study to our knowledge that addresses the effects of UV-B on pollinator attraction, more studies on different plant species and their pollinators are necessary to assess the generality of our results.

In summary, we found growth and flowering period of *B. rapa* and *B. nigra* to be decreased by increased UV-B exposure. Fitness, however, (estimated as seed number and seed quality) was generally elevated at the higher UV-B doses examined in our experiments. Offspring of UV-B exposed plants do not appear to suffer any major detrimental effects. Finally, neither floral traits nor attractiveness to pollinators was altered by increased levels of UV-B.

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