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Physiological and Yield Effects of Uniconazole on Winter Rape (*Brassica napus* L.)

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Abstract. Uniconazole at various concentrations on rape, at the three-leaf stage, was examined for physiologic and yield effects. Foliar sprays of 10, 25, and 50 mg/liter significantly reduced seedling height, and increased shoot width (stem width before elongation), number of green leaves, and total dry weight at transplanting. Chlorophyll content, superoxide dismutase and catalase activities, root oxidizability (capacity for root oxidation), and ethylene production were also increased. Additionally, the number of branches and pods/plant were increased; and a 7.4, 8.5, and 4.3% increase of seed yield over the controls was observed with treatments at 10, 25, and 50 mg/liter uniconazole, respectively. No significant effects were observed on plant maturity, the seed oil content, or the erucic acid and glucosinolate content. Total oil production significantly increased with 10, 25, and 50 mg/liter by 9.9, 10.6, and 6.8%, respectively, over the controls. These results suggested that uniconazole-induced high productivity was accompanied by increased levels of activities of various antioxidants, including superoxide dismutase and catalase, and by the improvement of root oxidizability and plant vigor.

Key Words. *Brassica napus* L.—Uniconazole—Physiology

Transplanting is the main method for producing oilseed rape (*Brassica napus* L.) in China. It has been shown that the seed yield is 20% higher using vigorous seedlings compared with weak seedlings. In recent years it has been difficult to have vigorous seedlings and concomitant seed yields because of substandard seed beds, high seeding rate, and ineffective seedling management (Zhou 1994). Therefore, chemical plant growth regulators are playing important roles in crop production, and they are increasingly being used to manipulate plant growth and yield (Nickell 1982). In recent years several triazole derivatives have been developed for use as either fungicides or plant growth regulators. Triadimefon, triadimenol, and S-3308 have been categorized as fungicides, whereas paclobutrazol and uniconazole have been developed as plant growth retardants (Fletcher et al. 1986). Triazole plant growth regulators induce a variety of morphological and biochemical responses in plants, including retarded shoot elongation, stimulated rooting, and protection from various environmental stresses, making them ideal candidates for rape production (Davis et al. 1988, Fletcher and Hofstra 1988). Paclobutrazol [(2*RS*, 3*RS*) -1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-yl) pentan-3-ol] is a plant growth retardant and has been used extensively to reduce seedling height and to prevent foot-high and weak seedlings in rape (Scarisbrick et al. 1985, Shen et al. 1991, Wu 1987, Zhou and Xi 1993). In China, about a quarter of the total rape acreage, i.e. 1.2 millions ha, is treated with paclobutrazol to raise dwarf rape seedlings (Wang 1993). However, paclobutrazol residues in rape plants and soil may cause environmental problems (exhibiting "secondary retardatory effect" on the succeeding crop if used continuously) (Xi et al. 1995). It is reported that uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] shows a much higher inhibitory effect on plant growth and development, and it degrades more easily in soil, and there are fewer residues in plants compared with paclobutrazol (Izumi et al. 1984, Wang et al. 1993a). The objective of this presentation is to report the physiological and yield effects of uniconazole on rape plants under field conditions.

Materials and Methods

Oilseed rape (*B. napus* L.) cv. 601 was examined. A 5% water-dispersible power of uniconazole (high effect triazole) was produced and provided by the United Chemical Factory of Zhangjiegang City, Jiangsu Province. The experiments were conducted at the university

Abbreviations: SOD, superoxide dismutase; CAT, catalase; NBT, nitro blue tetrazolium; TTC, red tetrazolium; IAA, indoleacetic acid.

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Table 1. Effect of uniconazole on seedling size of rape at transplanting.[a]

Uniconazole concentration (mg/liter)	Seedling height (cm)	Shoot width[b] (cm)	Green leaf[c] (no.)	Dry weight (g/plant)		
				Root	Shoot	Total
0(control)	29.3aA	0.50aA	4.8aA	0.153aA	0.850bAB	1.003abAB
5	27.0bAB	0.52abA	4.9abA	0.159abA	0.893bAB	1.052bAB
10	23.2cBC	0.55bcA	5.1abA	0.167bcA	1.013cB	1.180cB
25	22.0cdBC	0.55bcA	5.6cA	0.180cA	1.007cB	1.187cB
50	20.9dC	0.57cA	5.4bcA	0.171bcA	0.904bAB	1.075bAB
75	17.6eD	0.54bcA	5.2bA	0.164bA	0.862bAB	1.026abAB
100	15.2fD	0.56bcA	5.2bA	0.160abA	0.794aA	0.954aA

[a] All treatments were applied as foliar sprays on 8 November (at the three-leaf stage) at the rate of 750 liters of formulated solution/ha. Transplanting was carried out on 18 November (at the five-leaf stage). Within columns, means followed by the same capital and small letters are not significantly different at the 0.01 and 0.05 levels of probability, respectively, according to Duncan's New Multiple Range Test.

[b] Stem width before elongation.

[c] Excluding dead and yellow leaves.

farm (Hangzhou, 30°10′N, 120°12′E) during the 1994–1995 season and were based on the preliminary results with uniconazole during the 1993–1994 season. 601 rape seeds were sown on 5 October in a seedbed of a silt loam soil. Seven concentrations of uniconazole were made as follows: 0 (distilled water, control), 5, 10, 25, 50, 75, and 100 mg/liter. All treatments were applied as foliar sprays on 8 November (at the three-leaf stage) at the rate of 750 liters of formulated solution/ha. Seedlings were transplanted on 18 November (at the five-leaf stage) into the experimental plots in a randomized block design using three replicates. Each bed was 6.0 m long and 1.7 m wide, containing a total of 120 plants. Conventional cultivation methods were used during the growing period. All plants were harvested on 14 May (at which two out of three pods on plants began to yellow).

Ten plants taken from the central rows of each plot were used for physiological analyses and morphological measurements at time of seedling transplant. Leaf chlorophyll content was determined by the acetone/ethanol mixture assay method (Chen 1984). Leaf superoxide dismutase (SOD) activity was measured by the method of Zhu et al. (1990). Leaf tissue (500 mg) was homogenized at 4°C in 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrrolidone with a mortar and pestle. The homogenate was centrifuged at 15,000 ×*g* for 10 min, and the supernatant obtained was used as enzyme extract. SOD activity was assayed essentially as described by Dhindsa et al. (1981) by measuring the ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT). The 4-mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 77.12 μM NBT, 0.1 mM EDTA, 13.37 mM methionine, 0–10 μL of enzyme extract, and 100 μL of 80.2 μM riboflavin (riboflavin was added last). Leaf catalase (CAT) activity was analyzed by the hydrogen peroxide reduction method (Zhou and Lin 1995). Leaf extracts were treated with 5 mL of 0.1 N H_2O_2 and kept at 20°C for 5 min. Then 1 mL of 20% KI solution was added with 3 drops of 10% $(NH_4)_6Mo_7O_{24}$ solution and 5 drops of 1% starch solution. 0.02 N $Na_2S_2O_3$ was used to titrate the reaction solution until the disappearance of blue color. CAT activity was assayed by determining the rate of reduction of hydrogen peroxide during the given period.

Root oxidizability (capacity for root oxidation) was measured by the red tetrazolium (TTC) reduction method (Shen et al. 1991). The roots were washed free of soil, then 2.0 g of roots from each treatment was placed in a 25-mL test tube and sealed with a rubber stopper. The tubes were treated with 5 mL of 0.4% TTC solution and 5 mL of 1/15 M phosphate buffer (pH 7.0). After incubation for 3 h at 37°C, the tubes were treated with 2 mL of 2 N H_2SO_4. Then roots were ground with ethyl acetate (total of 10 mL) to extract red triphenylformazane. The absorbance of the extract was read at 485 nm. Ethylene evolution was determined using gas chromatography (Dong et al. 1983). Plants (excluding the roots) were put in a 60-mL culture tube sealed with a rubber stopper. The tubes were incubated for 2 h at 25°C, after which time a 1.0-mL sample of the headspace gas was removed with a hypodermic syringe and analyzed for ethylene using a gas chromatograph equipped with a 4- × 2,000-mm Al_2O_3 column (1.5% Apieson) and a hydrogen flame ionization detector.

Seedling height, shoot width (stem width before elongation), number of green leaves (excluding dead and yellow leaves), and plant dry weight were measured from ten random seedlings at transplanting. Plant height, stem width, branching position (distance between the cotyledon node and lowest primary branch), number of primary and secondary branches, pods/plant, seeds/pod, and seed weight were recorded from ten random plants at harvest. Seed yield of each plot was obtained manually from all the plants mixed together. Harvested seeds from the control and uniconazole-treated plants were analyzed for oil content (fatty acid extractor method), erucic acid content (gas chromatography), and glucosinolate content ($PdCl_2$ reaction assay (Zhou et al. 1993). Data from the three replications were pooled, and statistical inferences were made based on Duncan's New Multiple Range Test between control and uniconazole means.

Results

Uniconazole retarded seedling height (Table 1). No phytotoxicity was observed with any of the treatments. Leaf color became darker, and the seedlings became shorter and stronger within 5–7 days after uniconazole spraying. Shoot width and the number of green leaves at transplanting were increased by uniconazole (Table 1). The 10-, 25-, and 50-mg/liter uniconazole-treated plants had higher total dry weights, which were significantly increased by 17.6, 18.3, and 7.2%, respectively, over the controls. The 100 mg/liter uniconazole-treated plant had low shoot dry weight, which was significantly decreased by 6.6% over the control, and consequently, its total dry weight was also lower than the control.

Table 2. Effect of uniconazole on chlorophyll content and SOD and CAT activities of rape leaves at transplanting.[a]

Uniconazole concentration (mg/liter)	Chlorophyll content (mg/g FW)	SOD activity (unit/g FW/min)	CAT activity (H_2O_2 mg/g FW/min)
0(control)	1.68aA	58.90aA	30.65aA
5	1.69abA	60.34abA	31.60abA
10	1.74abA	63.95bcA	32.15abA
25	1.80bA	65.43cA	33.21bA
50	1.83bA	66.07cA	32.95bA
75	1.78abA	65.02cA	33.47bA
100	1.74abA	62.52abcA	31.85abA

[a] All treatments were applied as foliar sprays on 8 November (at the three-leaf stage) at the rate of 750 liters of formulated solution/ha. Transplanting was carried out on 18 November (at the five-leaf stage). Within columns, means followed by the same capital and small letters are not significantly different at the 0.01 and 0.05 levels of probability, respectively, according to Duncan's New Multiple Range Test.

Table 3. Effect of uniconazole on root oxidizability and leaf ethylene production of rape plants at transplanting.[a]

Uniconazole concentration (mg/liter)	Root oxidizability (TTC mg/g FW/h)	Leaf ethylene production (nL/g FW/h)
0(control)	0.441aA	0.178aA
5	0.462abA	0.204bA
10	0.481bcAB	0.302eC
25	0.506cAB	0.283eC
50	0.563dB	0.230cdAB
75	0.490bcAB	0.246dBC
100	0.471abAB	0.223cAB

[a] All treatments were applied as foliar sprays on 8 November (at the three-leaf stage) at the rate of 750 liters of formulated solution/ha. Transplanting was carried out on 18 November (at the five-leaf stage). Within columns, means followed by the same capital and small letters are not significantly different at the 0.01 and 0.05 levels of probability, respectively, according to Duncan's New Multiple Range Test.

Leaf chlorophyll content was increased after treatment with uniconazole (Table 2). The highest chlorophyll values were obtained using 25 and 50 mg/liter uniconazole and gave readings 7.1 and 8.9% greater than the controls. Uniconazole also increased the activities of SOD and CAT in leaves (Table 2). A better effect was achieved from 25-, 50-, and 75-mg/liter uniconazole treatments, where the SOD activity was increased by 11.1, 12.2, and 10.4%, and the CAT activity was increased by 8.4, 7.5, and 9.2%, respectively, over the controls.

The root oxidizability (capacity for root oxidation) was increased with uniconazole (Table 3). The 10-, 25-, 50-, and 75-mg/liter uniconazole-treated plants had higher root oxidizabilities, which were significantly increased by 9.1, 14.7, 27.7, and 11.1%, respectively, over the controls.

Foliar spray of uniconazole significantly increased ethylene production (Table 3). The endogenous ethylene evolution of 5-, 10-, 25-, 50-, 75-, and 100-mg/liter uniconazole treatments was significantly increased by 14.6, 69.7, 59.0, 29.2, 38.2, and 25.3%, respectively, over the controls.

Plant height at harvest was unaffected by uniconazole; no obvious differences in stem width and branching position (distance between the cotyledon node and lowest primary branch) were found between various treatments and the control (Table 4). The number of primary branches in 10-, 25-, and 75-mg/liter uniconazole-treated plants and of the secondary branches in 50-mg/liter-treated plants were significantly increased by 13.7, 12.3, 9.6, and 7.0%, respectively, over the controls.

The number of pods/plant was increased by uniconazole (Table 4). A better effect was obtained from 10- and 25-mg/liter uniconazole treatments, and the number of pods/plant was increased by 6.5 and 6.9%, respectively, over the controls. No significant difference in the number of seeds/pod and seed weight was observed between the treatments or controls. The seed yield of 10- and 25-mg/liter uniconazole treatments reached 1585.5 and 1602.0 kg/ha, respectively, a significant increase over the controls of 7.4 and 8.5%.

There was no increase in seed oil content from treated plants, but the oil yields of 10-, 25-, and 50-mg/liter treatments increased significantly by 9.9, 10.6, and 6.8%, respectively, over the controls (Table 5). No obvious difference of erucic acid and glucosinolate content of seeds was observed between the treatments and the control. In addition, the maturation of plants was not markedly affected.

Discussion

Uniconazole blocks gibberellin biosynthesis and retards growth in rice seedlings. Specifically, the reaction site of uniconazole was shown to be the three oxidation steps from kaurene to kaurenoic acid (Izumi et al. 1985). The inhibitory effects can be reversed using exogenous gibberellin (Mita and Shibaoka 1984). Uniconazole enhances IAA oxidase activity, thereby reducing endogenous IAA in rice seedlings (Wang et al. 1993a) and subsequently, weakening apical dominance and promoting initiation and growth of lateral branches. The present experiment showed that seedling height was significantly reduced, and the shoot width and number of primary and secondary branches were increased following treatments. These results were consistent with the previous reports of uniconazole on rice plants (Izumi et al. 1984, Wang et al. 1993b).

Ethylene inhibits plant height (Wareing and Phillips 1981), and the rate of ethylene evolution in uniconazole-treated rape seedlings was higher than in the controls.

Table 4. Effect of uniconazole on yield components and yield of rape.[a]

Uniconazole concentration (mg/liter)	Plant height (cm)	Stem width (cm)	Branching position[b] (cm)	Primary branch (no.)	Second branch (no.)	Pod/plant (no.)	Seed/pod (no.)	Seed weight (mg)	Seed yield (kg/ha)
0(control)	144.8NS[c]	1.76NS	35.4NS	7.3aA	5.7abA	328.5aA	16.1abA	3.40NS	1476.0aA
5	144.5	1.79	34.9	7.6abA	5.9abcA	329.7abA	16.5abA	3.38	1488.0abA
10	149.7	1.78	34.4	8.3cA	5.6aA	349.8bA	17.0bA	3.45	1585.5bcA
25	152.4	1.82	33.4	8.2cA	6.0bcA	351.2bA	16.3abA	3.51	1602.0cA
50	150.0	1.80	35.0	7.8abcA	6.1cA	338.5abA	16.9abA	3.48	1539.0abA
75	152.1	1.75	33.9	8.0bcA	5.5aA	339.6abA	15.8aA	3.54	1480.5abA
100	145.5	1.77	34.3	7.5abA	5.9abcA	327.8aA	16.3abA	3.39	1441.5aA

[a] All treatments were applied as foliar sprays on 8 November (at the three-leaf stage) at the rate of 750 liters of formulated solution/ha. Transplanting was carried out on 18 November (at the five-leaf stage). Within columns, means followed by the same capital and small letters are not significantly different at the 0.01 and 0.05 levels of probability, respectively, according to Duncan's New Multiple Range Test.
[b] Distance between the cotyledon node and lowest primary branch.
[c] NS, not significant at the 0.05 level of probability.

Table 5. Effect of uniconazole on rapeseed quality.[a]

Uniconazole concentration (mg/liter)	Oil content (% of seeds)	Oil yield[b] (kg/ha)	Erucic acid (% of fatty acids)	Glucosinolate (μmol/g meal)
0(control)	38.45NS[c]	510.8aA	48.40NS	108.4NS
5	38.58	516.7abA	49.00	107.9
10	39.34	561.4cA	48.25	110.2
25	39.20	565.2cA	48.51	108.7
50	39.38	545.5bcA	49.34	109.4
75	38.53	513.4abA	47.95	113.0
100	38.54	500.0aA	48.26	108.2

[a] All treatments were applied as foliar sprays on 8 November (at the three-leaf stage) at the rate of 750 liters of formulated solution/ha. Transplanting was carried out on 18 November (at the five-leaf stage). Within columns, means followed by the same capital and small letters are not significantly different at the 0.01 and 0.05 levels of probability, respectively, according to Duncan's New Multiple Range Test.
[b] Oil yield (kg/ha) = seed yield (kg/ha) × oil content (%) × 0.9.
[c] NS, not significant at the 0.05 level of probability.

Similar results in ethylene production were obtained from paclobutrazol-treated rape and rice (Zhang et al. 1988, Zhou et al. 1993). However, several studies reported that triazole compounds, including triadimefon and uniconazole, reduced ethylene production in cucumber, wheat, soybean, and mung bean seedlings (Abbas et al. 1989, Hofstra et al. 1989, Kraus et al. 1991). These results have suggested that a change in endogenous ethylene evolution is involved when triazole derivatives are applied to plants.

The present experiments indicated that the foliar sprays of uniconazole could delay degradation of SOD and CAT activities and decrease of the chlorophyll content of rape plants. These results were consistent with previous reports that triazole-induced stress tolerance and high productivity were due, at least in part, to increased antioxidant activity that reduced stress-related oxidative damage to cell membranes (Kraus and Fletcher 1994, Kraus et al. 1995, Upadhyaya et al. 1989). In addition, uniconazole has been reported to enhance nitrate reductase activity and plant photosynthetic rate and to increase soluble protein and total sugar content (Liu et al. 1993, Yang et al. 1994). The present experiments also indicated that uniconazole may promote root oxidizability and, therefore, improve root growth.

The physiological and yield effects of uniconazole foliar sprays at rape three-leaf stage varied with concentrations, and better effects were obtained using 10- and 25-mg/liter uniconazole treatments than 5- and 50-mg/liter treatments. Treatment at 100 mg/liter induced insignificant physiological effects or yield reduction. The effective concentration of paclobutrazol, now widely applied on rape, ranged from 100 to 200 mg/liter (foliar sprayed at the three-leaf stage) (Zhou et al. 1993). Thus, compared with paclobutrazol, low concentrations of uniconazole were required for inhibition of rape. Moreover,

uniconazole degraded more easily in soil, and there were fewer residues in plants and soil than with paclobutrazol (Wang et al. 1993a). Therefore, although it needs to be proved further, there appeared to be less secondary retardatory effect (retardation on the succeeding crop) with uniconazole, as is often the case with paclobutrazol; and from an environmental perspective, it was comparatively safer to apply uniconazole to raise vigorous seedlings.

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