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Effect of environmental conditions on plant growth regulator activity of fungicidal seed treatments of barley

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Summary

The triazole fungicide triadimenol is known to have plant growth regulator (PGR) activity in cereals when applied as a seed dressing. The effect of environmental conditions on PGR activity of triadimenol, other triazoles (flutriafol, prothioconazole, tebuconazole), the benzimidazole fuberidazole, imidazoles (imazalil, prochloraz), and the strobilurin fluoxastrobin on barley (*Hordeum vulgare*) was investigated using commercial seed dressings also including pyrimethanil (anilinopyrimidine) and triazoxide (benzotriazine), under controlled conditions. Irrespective of temperature or soil water content (SWC) triazole-containing seed treatments had a significant effect on the time and rate of plant emergence. Both triadimenol-containing products significantly reduced the length of subcrown internodes and resulted in reduced shoot length three weeks after sowing. Growth suppression was stronger under optimal environmental conditions (17 to 19 °C, 60 % SWC). Under suboptimal conditions – 9 to 10 °C and 40 % SWC, respectively – no differences in shoot length were detected five weeks after sowing, whereas under optimal conditions plant growth retardation was still significant. The flutriafol-containing product partly inhibited shoot elongation, but never affected dry mass accumulation and root growth. The strobilurin-containing seed dressing had no marked plant growth activities on seedling emergence, shoot length and subcrown internode, but slightly stimulated root growth under all environmental conditions. The results indicate varying PGR activities of triazole seed dressings in response to mixing partner and growth conditions and suggest an increased stress tolerance of seedlings treated with triadimenol, enabling barley to better cope with suboptimal environmental conditions.

Introduction

Seeds of crops are regularly dressed with fungicides to control seed- and soil-borne plant pathogens for uniform and healthy early plant development and ultimately, to maximize crop yield. Demethylation inhibitors (DMIs) of fungal sterol biosynthesis, particularly triazoles, imidazoles and pyrimidines, represent the most important group of modern systemic fungicides used for seed dressings (KUCK et al., 1995). In addition to fungicidal compounds, triazoles (e.g. uniconazole, paclobutrazole) are used also for plant growth regulation (PGR) (BUCHENAUER, 1995). Similarly numerous morphological and physiological side-effects in plants have been described for fungicidal triazole-derivatives in addition to their primary effect on sterol biosynthesis of fungi, primarily based on the inhibition of cytochrome P450-dependent enzyme activities (FÖRSTER et al., 1980a; FLETCHER et al., 1986; GAO et al., 1987; SIEFERT and GROSSMANN, 1996). Triazoles registered for their fungicidal activity in cereals exhibit growth retardation activity, e.g. tebuconazole and metconazole in oil seed rape (GILGENBERG-HARTUNG, 1999; KRUSE and VERREET, 2005).

Triazole seed treatments affect many aspects of plant growth and development. Triadimefon and triadimenol, early commercialized DMIs, result in growth inhibition of roots, shoots and coleoptiles of barley seedlings (FÖRSTER et al., 1980a). Retardation of primary leaf elongation has been reported in several crops (BUCHENAUER and RÖHNER, 1981; BUCHENAUER et al., 1984). PGR effects of triadimenol and triticonazole in wheat seedlings include reductions in the length of coleoptiles, primary leaves and subcrown internodes (ANDERSON, 1989; CAVARIANI et al., 1994; MONTFORT et al., 1996). Tebuconazole showed PGR activities in linseed and wheat seedlings (CAVARIANI et al., 1994; HACK, 1994). Seed treatments with propiconazole and diclobutrazole increased frost hardiness and tolerance of plants to drought and salt stress (BUCHENAUER et al., 1981). The sterol biosynthesis inhibitor imazalil restricted elongation of the subcrown internode and inhibited tillering of wheat seedlings (CHINN et al., 1980). The pyrimidine derivatives triarimol, nuarimol and fenarimol also exhibit PGR activities (SHIVE and SISLER, 1976; BUCHENAUER and RÖHNER, 1977; KONSTANTINIDOU-DOLTSINI et al., 1987).

The primary effect of fungicide seed treatments is restricted to early stages of plant growth, but side-effects on plant development may also influence yield formation in later growth stages. Furthermore, these physiological and morphological side-effects could improve the tolerance of barley subjected to various environmental stress conditions during seedling growth and development, because triazole applications gave an increased stress tolerance in various crops (FLETCHER et al., 2000). As the expansion of cropping area and climate change are associated with an increase in unfavourable conditions – temperature and soil conditions – in early growth stages, information is required whether fungicidal seed dressings including triazoles are suitable to promote crop development under suboptimal conditions. Therefore, it was investigated whether the triadimenol-containing seed dressing mixtures (+ imazalil + fuberidazole; Baytan UfB®; + prothioconazole + triazoxide; Baytan²®) exhibit stronger morphological and physiological side-effects on barley seedlings than a flutriafol-containing combination (+ prochloraz + pyrimethanil; Rubin®) or the mixture of the triazoles prothioconazole and tebuconazole combined with fluoxastrobin and triazoxide (EfA®). The environmental factors temperature, sowing depth and soil water content (SWC) were varied to assess their influence on incidence and magnitude of PGR effects.

Materials and methods

Seed and seed treatment

Pathogen-free seed of winter barley (*Hordeum vulgare* L., cultivar Fee) was treated with commercial fungicides as summarized in Tab. 1. Commercial products were used to guarantee optimum formulation of the fungicidal ingredients. They were applied at recommended application rates.

Plant cultivation

Barley kernels were sown in plastic containers (36 x 42 cm, height 18 cm) in four rows (row distance 10 cm) and a distance of 2.5 cm

Tab. 1: Composition and application rate of four seed dressing products.

No.	Active ingredient(s)	Chemical group	a.i.	Product	Application rate ^{A}	
			[g/l]		per 100 kg	[mg a.i./kg]
1	Triadimenol	Triazoles	75	Baytan UfB®	400 ml	300
	Imazalil	Imidazoles	10			40
	Fuberidazole	Benzimidazoles	9			36
2	Triadimenol	Triazoles	187.5	Baytan^2®	200 ml	375
	Prothioconazole	Triazoles	25			50
	Triazoxide	Benzotriazines	10			20
3	Flutriafol	Triazoles	16.7	Rubin®	250 ml	42
	Prochloraz	Imidazoles	38.5			96
	Pyrimethanil	Anilinopyrimidines	42			105
4	Prothioconazole	Triazoles	20	EfA®	200 ml	40
	Tebuconazole	Triazoles	3.75			8
	Fluoxastrobin	Strobilurins	37.5			75
	Triazoxide	Benzotriazines	10			20

^{A} on barley seed

within the rows, resulting in 48 seeds per container. In experiments on the effect of growth temperature and sowing depth sand (0 - 2 mm) was used as substrate; a mixture of sand and sieved C-horizon (≤ 0.6 cm) (ratio 1 : 1) was utilized in experiments on the influence of soil water content. Plants were grown in an univariate experimental design (I) at 17 - 19 °C and 9 - 10 °C (60 % SWC, 5 cm sowing depth (SD)), (II) at 40 % SWC and 60 % SWC (17 - 19 °C, 5 cm SD), and (III) with 3 cm SD and 5 cm SD (17 - 19 °C, 60 % SWC) under controlled conditions for 35 days. Plants were illuminated with at least 300 µmol PAR m^{-2} s^{-1}, 14 hours per day. Each treatment consisted out of four replicates of 24 plants (half container) and the containers with different treatments were distributed randomly under the respective growth conditions.

Plants were fertilized with a solution containing $(NH_4)_2SO_4$ (0.566 g kg^{-1} soil), K_2SO_4 (0.3 g kg^{-1}), KH_2PO_4 (0.193 g kg^{-1}) and $MgSO_4$ (0.507 g kg^{-1}). Each pot also received 30 ml of each of the following micronutrients: $MnSO_4$, 12.3 g l^{-1}, $ZnSO_4$, 17.6 g l^{-1}, $CuSO_4$, 15.72 g l^{-1}, H_3BO_3, 5.72 g l^{-1}, $(NH_4)_6Mo_7O_{24}$, 1.84 g l^{-1}, and $FeSO_4$, 99.55 g l^{-1}. Fertilization was split into three applications ten, fifteen and twenty days after sowing.

Control of soil water content (SWC)

Plants were irrigated as necessary to maintain optimal plant development. For experiments using specific SWCs, Watermark water potential sensors (Spectrum Technologies, Inc., Plainsfield, USA) were used for SWC measurements. Sensors were placed into the centre of each container during filling with the soil matrix which was adjusted with water to the designated SWC. Before and during the experiments, electrical resistance of each container was measured with an ohmmeter (Pontavi Wh 2, Hartmann and Braun, Frankfurt, Germany) at least once a day and compared with resistance values received from a calibration line of different SWCs in the lab.

Assessment of morphological and physiological parameters

The number of emerged seedlings was counted every day from the first sign of emergence until all germinated seedlings had emerged. Mean emergence was calculated by counting the emerged plants in each of all sowing rows of every treatment.

For the assessment of the following parameters 5 plants per replicate were selected randomly (n = 20). Shoot length, i.e. the distance between soil-surface and the tip of the longest leaf was measured 21 and 35 days after sowing. The length of the subcrown internode was determined 35 days after sowing.

The chlorophyll concentration of primary leaves was determined using a Minolta SPAD 502® chlorophyll meter (Minolta, Munich, Germany). The SPAD-Meter (**S**oil **P**lant **A**nalysis **D**evelopment) measures the greenness of leaf tissue (ratio 650 nm to 940 nm). Meter readings are given in SPAD-values indicating the relative chlorophyll concentration. SPAD-values were measured as an average of 5 leaves per replicate at the same date (n = 20).

For dry matter determination 5 shoots per replicate were harvested 35 days after sowing by cutting the seedlings above the soil surface. Shoots were dried first at room temperature and then at 105 °C for 24 h (n = 20). To determine the root dry mass after 35 days of plant growth, at least 15 intact root systems were removed from the soil by washing. Root systems were dried first at room temperature and then at 105 °C for 24 h. Root characteristics were determined using the WinRhizo system (Regent Instruments, Quebec, Canada). Root systems images of single plants were generated using a digital flatbed scanner (AGFA SnapScan 1236) according to the manufacturer's instructions. Root samples were placed in clear plastic trays, filled with water on the scanner bed. The scanner was equipped with a transmitted light source in the lid, so light came from above the roots during the scanning process. Images were analyzed using the root analysis software WinRhizo. It calculated morphological root measurements based on Tennant's statistical line intersect method (Tennant, 1975). Root samples were analyzed for root length, average diameter and volume.

Statistical analysis

The statistical analysis was performed using the software programme SPSS (Vers. 12, Munich, Germany). One-way ANOVA ($p \leq 0.05$) was used to detect differences between means. In case of significant differences among treatments, the Tukey test was used to differentiate treatments means. In figures, significant differences among treatments are shown by different letters.

Results

Effect of temperature on plant growth regulator activity

Plant emergence

At 17 to 19 °C, the optimal growth temperature, seeds treated with triadimenol-containing products as well as the mixture of triazoles and strobilurin gave emergence rates similar to untreated seeds; only the flutriafol-containing treatment had a marked effect on the emergence rate (Tab. 2). At lower temperature, only the strobilurin-containing mixture had no significant effect on plant emergence. All seed treatments, however, delayed the time of emergence by at least one day; the combination triadimenol + fuberidazole as well as flutriafol + prochloraz delayed emergence by two days (Fig. 1). At 17 to 19 °C, only the dressings including triadimenol and flutriafol delayed emergence, the other triazoles had no significant effect under optimal temperatures.

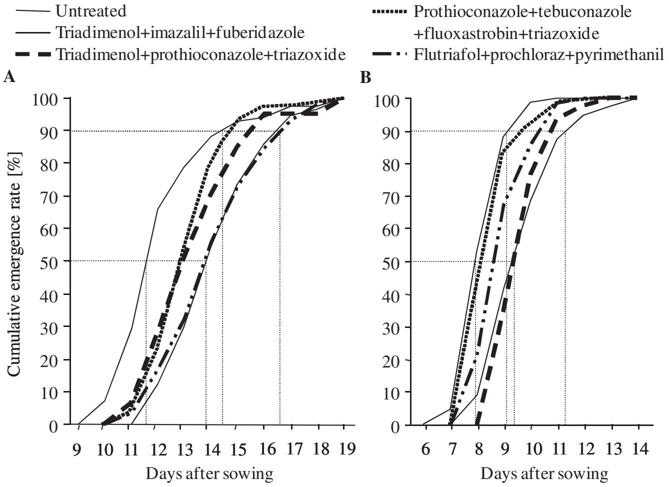

Fig. 1: Effect of growth temperature on the emergence of barley following fungicidal seed treatments. **A**, 9 - 10 °C; **B**, 17 - 19 °C (dotted lines indicate 50 % and 90 % emergence, respectively).

Shoot length

Both triadimenol seed dressings significantly retarded shoot length of 21 day-old seedlings grown at 17 to 19 °C and 9 to 10 °C, respectively, whereas the two other mixtures had no significant effect on plant height (Fig. 2). Differences between untreated and triadimenol-treated seedlings were less pronounced at 9 to 10 °C than at 17 to 19 °C. After growth for five weeks under suboptimal conditions, retardation effects on shoot length could not be detected any longer for any seed dressing (Fig. 2). Barley treated with triadimenol + prothioconazole was even significantly taller than that treated with the strobilurin-containing product. At 17 to 19 °C, shoots of 35 day-old barley treated with triadimenol dressings still were significantly shorter than the other treatments except for plants treated with the flutriafol-containing mixture.

Shoot dry matter production

After 35 days at 9 to 10 °C, no significant differences in shoot dry mass were detected among treated and untreated seedlings (Fig. 2). Compared to untreated barley, the treatment including fluoxastrobin significantly increased shoot dry mass production under optimal temperature. Seed treatments with triadimenol and flutriafol had no marked effect at 17 to 19 °C.

Tab. 2: Influence of environmental conditions and seed treatments on mean emergence of barley seedlings per seed row (max. 12).

Environment		Seed dressing				
		Untreated control	Triadimenol + imazalil + fuberidazole	Triadimenol + prothioconazole + triazoxide	Prothiocon. + tebucon. + fluoxastr. + triazoxide	Flutriafol + prochloraz + pyrimeth.
Temperature	9 - 10 °C	10.6^{a}	7.1^{b}	7.6^{b}	9.6^{a}	7.4^{b}
	17 - 19 °C	10.6^{a}	9.6^{ab}	9.9^{ab}	10.4^{a}	8.8^{b}
Soil water	40 %	10.1^{a}	8.3^{b}	8.8^{b}	10.6^{a}	8.8^{b}
content	60 %	10.6^{a}	8.3^{c}	9.4^{bc}	9.8^{ab}	8.8^{bc}
Sowing	3 cm	11.1^{a}	10.0^{a}	9.9^{a}	11.0^{a}	10.4^{a}
depth	5 cm	11.9^{a}	9.5^{b}	9.1^{b}	10.3^{b}	9.6^{b}

Same letters in a row indicate values not significantly different ($p \leq 0.05$; Tukey test)

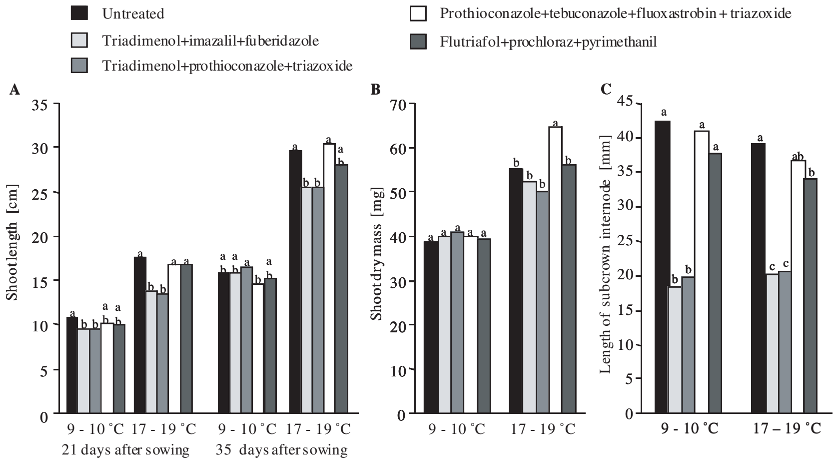

Fig. 2: Effect of growth temperature on plant growth regulator activities of fungicidal seed dressings on shoot length (**A**), dry matter production (**B**) and length of subcrown internodes (**C**) of barley. Values with same letters are not significantly different ($p \leq 0.05$, Tukey test).

Length of subcrown internode

Independent of temperature conditions, both triadimenol-containing seed dressings substantially inhibited the elongation of subcrown internodes as compared to the other treatments. At 17 to 19 °C, flutriafol + prochloraz reduced the length of subcrown internodes significantly when compared to control plants. Nevertheless, the elongation was affected to a much lesser extent than by the triadimenol-containing seed dressings (Fig. 2).

Effect of soil water content on plant growth regulator activity of fungicides

Plant emergence

All seed treatments containing DMI ingredients significantly reduced the rate of seedling emergence under both SWCs, except for the strobilurin-containing dressing which had no marked effect as compared to untreated barley (Tab. 2). With 40 % SWC, seed treatments significantly delayed barley emergence, except for the strobilurin-containing product (Fig. 3). In later stages, all treated seeds came up to similar emergence rates, not significantly different from that of untreated barley. With optimal SWC, barley emergence was faster from untreated seeds than from dressed seeds. Triadimenol + imazalil + fuberidazole and the fluoxastrobin product delayed emergence similarly, whereas the other two treatments slowed down the rate of emergence to a greater extent (Fig. 3).

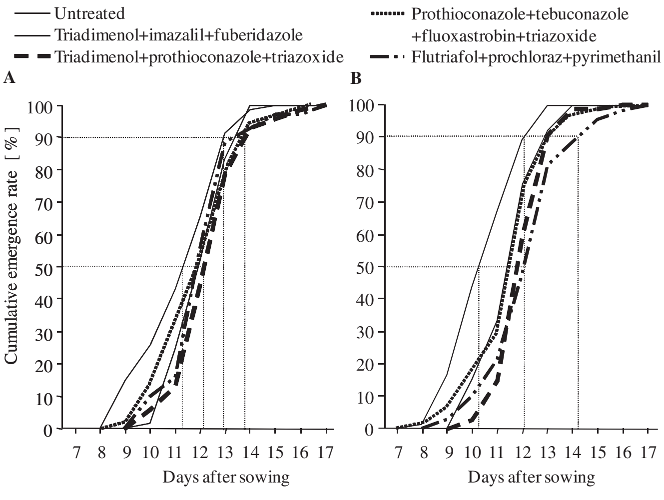

Fig. 3: Effect of soil water content (SWC) on the emergence of barley dressed with various fungicides. **A**, 40 % SWC; **B**, 60 % SWC (dotted lines indicate 50 % and 90 % emergence, respectively).

Shoot length

Three weeks after sowing, barley dressed with triadimenol-containing products and grown at 60 % SWC was severely stunted (Fig. 4). Shoot elongation of the other treatments was affected to a much lesser extent. Triadimenol + prothioconazole significantly restricted shoot elongation also under dry soil conditions. After five weeks of growth in dry soil no significant differences were observed among treatments (Fig. 4). When grown at optimal SWC of 60 % barley plants treated with triadimenol + prothioconazole or flutriafol + prochloraz were significantly shorter than untreated plants. The other seed dressings resulted in low growth suppression when compared to untreated plants.

Shoot dry matter production

In all treatments, low SWC (40%) caused a marked reduction in shoot dry mass compared to 60 % SWC; the difference was more pronounced than for shoot length (Fig. 4). Five weeks after seeding no significant differences were detected among treatments. In contrast, barley treated with triadimenol-containing seed dressings and grown under optimal SWC produced significantly less dry matter than untreated ones. Shoot dry matter of barley treated with triadimenol + prothioconazole was also significantly lower than that of plants grown from seeds dressed with flutriafol + prochloraz and prothioconazole + tebuconazole + fluoxastrobin, respectively.

Length of subcrown internode

Under both SWCs, triadimenol-containing seed dressings significantly inhibited elongation of the subcrown internode. The length of subcrown internodes of seedlings developing from the other treatments was similar to that of untreated plants (Fig. 4).

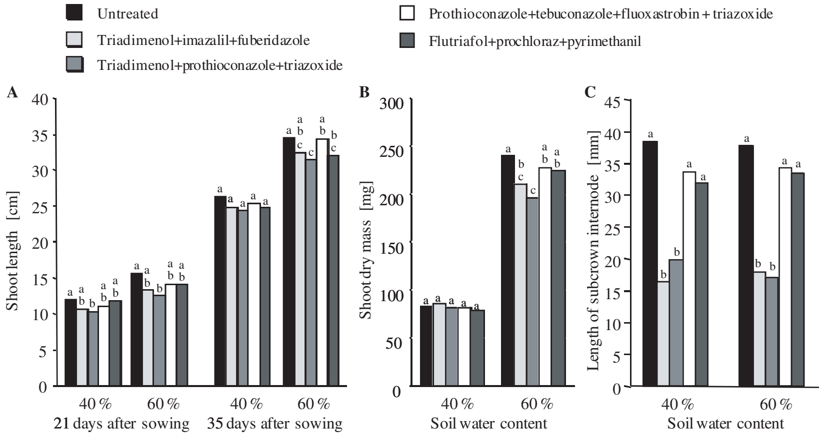

Fig. 4: Effect of soil water content (SWC) on plant growth regulator activities of fungicidal seed treatments on shoot length (**A**), dry matter production (**B**) and length of subcrown internode (C) of barley. Values with same letters are not significantly different ($p \leq 0.05$, Tukey test).

Effect of sowing depth on plant growth regulator activity of fungicides

Plant emergence

With 3 cm sowing depth (SD), no fungicide treatment had a marked effect on the rate of seedling emergence. Sowing at 5 cm depth, however, resulted in a significant reduction of barley emergence for all treatments (Tab. 2). Both triadimenol-containing treatments delayed emergence of barley sown at 3 cm depth at least by one day. Seedlings of the other treatments emerged slightly faster, but never reached the time of emergence of untreated barley. With 5 cm SD, seed treatments led to a significant delay in emergence. Especially triadimenol + imazalil + fuberidazole, but also the flutriafol-containing and the other triadimenol-containing dressings delayed seedling emergence stronger than the strobilurin-containing treatment (Fig. 5).

Shoot length

With 3 cm sowing depth, all treatments except for the flutriafol-containing product resulted in a significant reduction of shoot length 21 days after seeding (Fig. 6). Inhibition of shoot length from the combination triadimenol + prothioconazole was even significantly stronger than from the strobilurin-containing mixture. With 5 cm sowing depth, barley dressed with triadimenol and flutriafol products, respectively, was significantly shorter than plants from the other treatments. Five weeks after sowing at 3 cm depth no significant differences among untreated and treated barley could be detected any longer (Fig. 6). Shoot length of barley dressed with triadimenol-containing products planted at 5 cm depth was still significantly reduced 35 days after seeding.

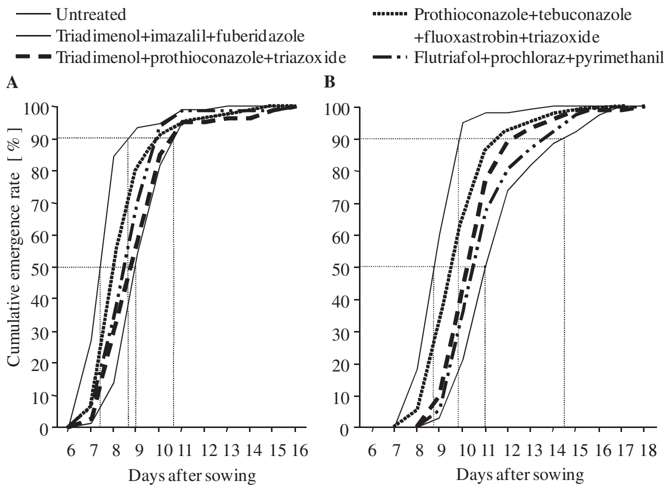

Fig. 5: Effect of sowing depth on the emergence of barley dressed with various fungicides. **A**, 3 cm sowing depth; **B**, 5 cm sowing depth (dotted lines indicate 50 % and 90 % emergence, respectively).

Shoot dry matter production

With both sowing depths, no seed treatment significantly influenced dry matter production of barley seedlings (Fig. 6). Shallow seeding slightly favoured dry matter accumulation in all treatments.

Length of subcrown internode

Independent of SD, triadimenol-containing products markedly reduced the elongation of subcrown internodes as compared to the other treatments (Fig. 6). Deeper seeding resulted in a subcrown internode generally 15 mm longer than for barley from seeds placed at 3 cm depth.

Effect of environmental conditions and fungicide seed treatments on chlorophyll content

Due to large plant-to-plant variability significant differences in chlorophyll concentration between untreated and fungicide-treated seedlings were not detected, except for triadimenol-containing seed treatments which caused either a significant or slight increase in chlorophyll content when compared to untreated plants (Tab. 3). A marked effect of environmental conditions on the side-effect of seed treatments on chlorophyll concentration could not be detected.

Effect of environmental conditions and fungicides on morphology and biomass of roots

Root length

No seed treatment exhibited a significant plant growth activity on root length (Tab. 4). At 9 to 10 °C, root length of seedlings treated with triadimenol and flutriafol, respectively, was significantly shorter

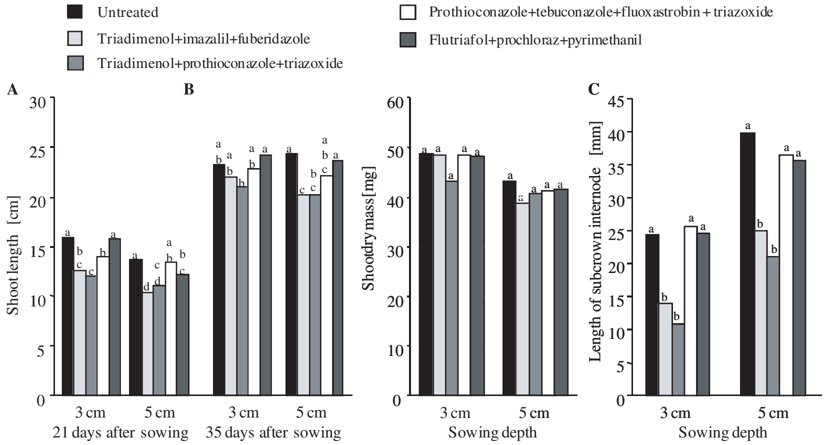

Fig. 6: Effect of sowing depth on plant growth regulator activities of fungicidal seed dressings on shoot length (A), dry matter production (B) and length of subcrown internodes (C) of barley. Values with same letters are not significantly different ($p \leq 0.05$, Tukey test).

Tab. 3: Effect of environmental conditions and seed treatments on relative chlorophyll concentration of barley leaves measured by SPAD-meter readings 21 and 35 days after sowing.

Seed dressing	Environmental condition			
	21 days after sowing		35 days after sowing	
Temperature:	9 - 10 °C	17 - 19 °C	9 - 10 °C	17 - 19 °C
Untreated control	41.6 ^{a}	42 ^{a}	47.8 ^{a}	37.8 ^{a}
Triadimenol + imazalil + fuberidazole	41.9 ^{a}	44 ^{a}	48.1 ^{a}	41.7 ^{a}
Triadimenol + prothiocon. + triazoxide	43.8 ^{a}	43.3 ^{a}	48.5 ^{a}	41.4 ^{a}
Prothiocon. + tebucon. + fluoxastr. + triaz.	40.8 ^{a}	43.1 ^{a}	49.2 ^{a}	38.7 ^{a}
Flutriafol + prochloraz + pyrimethanil	40.5 ^{a}	43.2 ^{a}	47.9 ^{a}	40.4 ^{a}
Soil water content:	40 %	60 %	40 %	60 %
Untreated control	38.1 ^{ab}	42.1 ^{a}	41.7 ^{b}	40.9 ^{ab}
Triadimenol + imazalil + fuberidazole	41.7 ^{a}	42.3 ^{a}	44.8 ^{a}	41.5 ^{ab}
Triadimenol + prothiocon. + triazoxide	38.4 ^{ab}	42.4 ^{a}	41.5 ^{b}	41.1 ^{ab}
Prothiocon. + tebucon. + fluoxastr. + triaz.	37.7 ^{ab}	44.8 ^{a}	42.9 ^{ab}	42.1 ^{a}
Flutriafol + prochloraz + pyrimethanil	35.9 ^{b}	41.9 ^{a}	41.6 ^{b}	39.2 ^{b}
Sowing depth:	3 cm	5 cm	3 cm	5 cm
Untreated control	44.8 ^{a}	43.6 ^{a}	39.4 ^{cd}	40 ^{b}
Triadimenol + imazalil + fuberidazole	47.3 ^{a}	47 ^{a}	44.9 ^{a}	44.7 ^{a}
Triadimenol + prothiocon. + triazoxide	44.7 ^{a}	47.6 ^{a}	43.6 ^{ab}	44.8 ^{a}
Prothiocon. + tebucon. + fluoxastr. + triaz.	44.5 ^{a}	46.1 ^{a}	38.9 ^{d}	41.3 ^{b}
Flutriafol + prochloraz + pyrimethanil	45.5 ^{a}	44.7 ^{a}	41.9 ^{bc}	40.7 ^{b}

Same letters in a column indicate values not significantly different ($p \leq 0.05$; Tukey test)

than that of the strobilurin-containing treatment. Except for 60 % SWC, seed treatments with this product slightly stimulated root growth.

Diameter of roots

No fungicide seed dressing had a significant side-effect on average root diameter. Except for 40 % SWC, roots of treated and untreated barley seedlings had similar diameters 35 days after sowing. Under dry soil conditions, roots of barley dressed with strobilurin or flutriafol-containing products were significantly thicker than roots of untreated and triadimenol + prothioconazole-treated plants, respectively. Significant differences between the root diameters of treated seedlings occurred also for plants grown at 9 to 10 °C and 17 to 19 °C, respectively.

Tab. 4: Effect of environmental conditions and seed treatments on root morphology and root dry weight of barley 35 days after sowing.

Seed treatment	Environmental conditions					
	Growth temperature		Sowing depth		Soil water content	
	9 -10 °C	17-19 °C	3 cm	5 cm	40 %	60 %
	Root length [cm]					
Untreated control	175.1 ^{ab}	266.9 ^{a}	286.6 ^{a}	284.3 ^{a}	473.1 ^{a}	510.8 ^{a}
Triadimenol + imazalil + fuberid.	161.1 ^{ab}	273.4 ^{a}	272.8 ^{a}	290.4 ^{a}	538.6 ^{a}	436.3 ^{a}
Triadimenol + prothioc. + triaz.	139.1 ^{b}	289.0 ^{a}	263.9 ^{a}	255.6 ^{a}	507.7 ^{a}	429.2 ^{a}
Prothioc. + tebuc. + fluoxa. + triaz.	200.8 ^{a}	335.0 ^{a}	339.8 ^{a}	351.4 ^{a}	556.0 ^{a}	502.0 ^{a}
Flutriafol + prochloraz + pyrimeth.	145.2 ^{b}	309.3 ^{a}	301.3 ^{a}	322.4 ^{a}	494.7 ^{a}	447.5 ^{a}
	Root dry-matter [mg]					
Untreated control	11.7 ^{ab}	9.8 ^{b}	39.0 ^{a}	37.3 ^{a}	30.6 ^{a}	39.9 ^{a}
Triadimenol + imazalil + fuberid.	11.3 ^{ab}	10.0 ^{b}	34.5 ^{a}	33.1 ^{a}	30.6 ^{a}	29.2 ^{a}
Triadimenol + prothioc. + triaz.	10.6 ^{b}	11.2 ^{b}	35.3 ^{a}	35.1 ^{a}	32.6 ^{a}	24.0 ^{a}
Prothioc. + tebuc. + fluoxa. + triaz.	12.5 ^{a}	14.2 ^{a}	39.5 ^{a}	39.1 ^{a}	30.2 ^{a}	38.2 ^{a}
Flutriafol + prochloraz + pyrimeth.	9.9 ^{b}	11.2 ^{b}	38.3 ^{a}	35.8 ^{a}	29.1 ^{a}	25.1 ^{a}
	Root diameter [mm]					
Untreated control	0.23 ^{ab}	0.29 ^{ab}	0.35 ^{a}	0.37 ^{a}	0.24 ^{c}	0.26 ^{a}
Triadimenol + imazalil + fuberid.	0.23 ^{b}	0.31 ^{a}	0.36 ^{a}	0.38 ^{a}	0.26 ^{abc}	0.29 ^{a}
Triadimenol + prothioc. + triaz.	0.28 ^{a}	0.29 ^{ab}	0.37 ^{a}	0.37 ^{a}	0.25 ^{bc}	0.29 ^{a}
Prothioc. + tebuc. + fluoxa. + triaz.	0.23 ^{b}	0.26 ^{b}	0.34 ^{a}	0.36 ^{a}	0.28 ^{a}	0.28 ^{a}
Flutriafol + prochloraz + pyrimeth.	0.24 ^{ab}	0.30 ^{a}	0.33 ^{a}	0.35 ^{a}	0.27 ^{ab}	0.29 ^{a}

Same letters in a column indicate values not significantly different ($p \leq 0.05$; Tukey test)

Root biomass

Under both temperature conditions and sowing depths, seedlings treated with the strobilurin-containing product produced the highest root dry matter (Tab. 4). At 17 to 19 °C, the dry matter was significantly higher than that of all other treatments. At low temperature, no significant differences could be detected between untreated and fungicide-treated barley seedlings; root dry matter of barley grown from seeds treated with the strobilurin product, however, was significantly higher than that of triadimenol- and flutriafol-treated plants. For both, SD and SWC, no significant differences could be detected among treatments.

Discussion

Composite seed dressing products containing triadimenol had similar PGR activities as described for the use of triadimenol alone. Reduction and delay of plant emergence, inhibition of shoot growth and the reduction in subcrown internode length are commonly observed morphological side-effects following triadimenol applications (Frohberger, 1978, Förster et al., 1980a, Cavariani et al., 1994; Montfort et al., 1996). The strong inhibition of root growth described by Förster et al. (1980a) could not be confirmed, probably because of the different stages of plant development in which the root length were examined in both studies. A compensation of the initial growth inhibition in later development stages would be plausible. An increase in leaf thickness in response to a reduction of leaf area from triazole derivatives has been reported by Benton and Cobb (1995). Additional layers of palisade mesophyll cells per unit area described for triadimenol-treated wheat (Gao et al., 1987) may be involved in these effects and may also explain the slight increase in chlorophyll concentration of barley plants. The results revealed that shoot biomass of seedlings was affected less than shoot elongation, maybe due to increased width and thickness of leaves, often reported as a side-effect of triazole applications (Buchenauer and Röhner, 1981; Förster et al., 1980b; Buchenauer et al., 1984; Gao et al., 1987; Benton and Cobb, 1995).

Lower PGR activities of seed dressings containing flutriafol and prochloraz and prothioconazole and tebuconazole, respectively, may be attributed to a lower affinity of the different triazole- and imidazole-derivatives to the cytochrome P-450 dependent enzymes involved in gibberellin biosynthesis. Shoot length and subcrown internode length of seedlings from kernels treated with these products were reduced only slightly. These plant growth parameters support the previous assumption that also other triazoles and imidazoles retard early plant growth. The concentration of active ingredients in seed treatments as well as *in planta* and their affinity to the cytochrome P-450 dependent enzymes, however, may have an impact on the magnitude of plant growth activities. Cavariani et al. (1994) reported that plant growth retardation from tebuconazole was lower than that from triadimenol at the same concentration.

Montfort et al. (1996) attributed the reduced emergence of azole-treated wheat seedlings to the reduction of subcrown internode length, the results, however, revealed that flutriafol + prochloraz significantly reduced the emergence rate without affecting the length of subcrown internodes. Reduced coleoptile elongation particularly described as an effect of triadimenol treatments (Frohberger 1977, 1978), probably had a stronger effect on seedling emergence of barley dressed with triadimenol than on plants treated with flutriafol + prochloraz. The emergence of primary leaves, produced from retarded coleoptiles and additionally exposed to suboptimal environmental conditions is more complicated. This assumption is in accordance with lower emergence of seeds treated with triadimenol and flutriafol products at lower sowing depth or grown at low temperature. Lindstrom et al. (1976) and DeJong and Best (1979) concluded that the rate of coleoptile elongation before seedling emergence is the most important determinant of crop establishment. Factors that reduce the rate and magnitude of coleoptile elongation increase the risk of stand failure (Allan et al., 1962; Burleigh et al., 1965; Lindstrom et al., 1976; Sunderman, 1964). A disturbed geotropism may also contribute to a reduced emergence rate (Förster et al., 1980b). Primary leaves of seedlings from triadimenol-treated kernels sometimes emerged some days later than the majority, exhibiting the shape of a horseshoe, in which the tip of the primary leaves still remained reversed in the soil.

Environmental conditions, particularly suitable sowing conditions like moderate SWC and ambient temperature, enhanced and extended PGR activities of fungicidal seed treatments. Shoot growth regulating activities of both triadimenol seed treatments were markedly stronger than at lower temperature or low SWC. Only under optimal growth conditions the effect was still detectable 35 days after sowing. Higher transpiration rates at 60 % SWC and 17 to 19 °C, respectively, based on a high air to leaf vapour pressure deficit, are likely to enhance uptake and transfer of azoles into stems and leaves. In contrast, low transpiration under suboptimal conditions resulted in a lower uptake and transfer of active ingredients, which influenced shoot development to a much lesser degree.

The length of subcrown internodes indicated that temperature and soil water content have no effect on the magnitude of PGR activity of triadimenol-containing seed dressings and that both factors have no marked effect on crown depth. Shallow sowing, however, decreased the length of subcrown internodes, and triadimenol-containing seed treatments significantly reduced the distance even more. Huang and Taylor (1993) reported that the formation of subcrown internodes in wheat seedlings is markedly influenced by sowing depth, whereas Webb and Stephens (1936) and Dofing and Schmidt (1984) concluded that the crown position is primarily affected by soil temperature. Our results indicate that, in addition to sowing depth fungicidal seed dressings exhibit a strong effect on the length of subcrown internodes. The determination of the crown position takes place early after seed germination and plant emergence, respectively (Taylor and McCall, 1936). Uptake of high triadimenol amounts by the roots within the first days of plant development lead to a strong inhibition of the subcrown internode length.

In addition to their fungicidal and plant growth retardation effects, triazoles have been reported to enhance tolerance of plants to environmental stress factors (Fletcher et al., 2000). The smaller growth inhibitory effects of triazole-containing seed treatments, observed under less favourable growth conditions (low temperature, low SWC), may be also explained as improved stress response of treated barley seedlings. Partial closure of stomata and a transient rise in abscisic acid levels reported for triadimefon-treated beans (Asare-Boamah et al., 1986) as well as the maintenance of increased antioxidant activity by uniconazole in maize (Li et al., 1998) are physiological effects of triazoles which may improve growth under stress conditions. Sairam et al. (1995) reported that triadimefon increased tolerance of wheat to drought stress by reducing transpiration and increasing the relative water content as well as membrane stability. As shorter barley seedlings with low levels of gibberellins were highly tolerant to stress, Sarkar et al. (2004) concluded that reduced gibberellin levels coupled with reduction in height are important for the induction of stress tolerance. Shorter seedlings due to triadimenol treatments most probably possess higher tolerance to environmental stresses. Consequently, triadimenol-treated seedlings may show increased survival during periods of drought or low temperatures, but, due to the lesser inhibition of gibberellic acid biosynthesis, stress tolerance induced by fungicidal seed treatments is likely to be lower than from commercial PGR like paclobutrazole.

This study indicates that triadimenol-containing seed treatments possess stronger PGR activities than seed treatments containing other triazoles or imidazoles. Strobilurins also known to influence plant development and yield formation of cereals are reported to interfere with PGR metabolism and to have an anti-stress activity (Grossmann and Retzlaff, 1997). As part of composite seed dressings they seem to initiate other metabolic modifications than triazoles resulting in a kind of compensation. The intensity of PGR is modified by environmental conditions the seedlings are subjected to in early growth stages. In addition to their fungicidal activities, triadimenol-containing seed treatments could contribute to an increased tolerance to abiotic stress factors enabling barley seedlings to better cope with suboptimal growth conditions.

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