Use of a Complexation of Tebuconazole with *β*-Cyclodextrin for Controlling Foot and Crown Rot of Durum Wheat Incited by *Fusarium culmorum*

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The methodology for the inclusion of tebuconazole (TBC) in *β*-cyclodextrin (*β*CD), spectroscopic characterization of the inclusion complex, and its activity for the control of a major soilborne disease of wheat caused by *Fusarium culmorum* are reported. Controlled release measured by chemical shift of the diagnostic protons H_3 and H_5 of *β*CD confirmed stability of the complex at the solid state and in aqueous solution. Greenhouse and field experiments were conducted on durum wheat (*Triticum durum* cv. Prometeo) sown in substrate or in soil artificially infested with a virulent strain of *F. culmorum*. The inclusion complex *β*CD−TBC, applied as seed dressing in combination or not with carboxymethylcellulose, reduced the disease incidence caused by *F. culmorum* and improved grain yield, showing effects that were generally comparable to those observed upon application of a commercial formulation of TBC. In the field experiment, only seed treatment with the inclusion complex *β*CD−TBC allowed yield that was not different from that obtained from the uninoculated control. These results prove that by the use of the *β*CD−TBC complex it is possible to obtain release of TBC and bioavailabilty of the fungicide without compromising its effectiveness.

KEYWORDS: Tebuconazole; *β*-cyclodextrin; inclusion complex; wheat; crown and foot rot; *Fusarium culmorum*

INTRODUCTION

Crown and foot rot of wheat is an important soilborne disease caused by several species of filamentous fungi (*1*). *Fusarium culmorum* (W.G. Smith) Sacc. is one of the most common incitants of this disease worldwide (*2−4*). The severity of crown and foot rot caused by *F. culmorum* is greater in dry soils and at high temperature (*5, 6*), conditions that are present in the central and southern parts of Italy, where durum wheat is commonly grown. The main symptoms of this disease are seedling death before or soon after emergence, brown lesions on the basal portion of the stem, and tiller abortion and formation of whiteheads, resulting from premature death of the plant. Whiteheads may contain shriveled grain or no grain at all. As a consequence, significant yield loss is reported.

The control of crown and foot rot is difficult and relies on the adoption of a preventive measure, including crop rotation, use of tolerant cultivars, management of crop residues, and reduced use of nitrogen fertilization and seed dressing with fungicides.

Chemical control by seed treatment represents an efficient means of control, but it is usually limited to the early stages of the wheat cycle, because the fungicides do not maintain their efficiency for a longer period. This may be due to different factors, such as environmental conditions, physical soil properties, or rapid degradation of the active ingredient by the soil microflora. Regardless of the systemic properties of the fungicide, methods are needed to improve the slow release of the delivered compound, so that the plant organs may be protected for a longer phase.

The molecular inclusion of pesticides offers several advantages over conventional agrochemical formulations: a constant level of the active ingredient is released, providing enhanced efficacy, reduced mammalian toxicity and phytotoxicity, and an increased solubility of water-insoluble compounds. The latter

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Control of Foot and Crown Rot of Durum Wheat

J. Agric. Food Chem., Vol. 54, No. 2, 2006 **481**

aspect is particularly important, because it allows more effective penetration of the fungicide.

β-Cyclodextrin (βCD) is a nontoxic, inexpensive, and water-soluble biopolymeric matrix already used in various industrial, medical, and agricultural applications for a slow delivery of the active chemical agent (7−11). It is a torus-like amphipathic macro-ring built up from glucopyranose units with a lipophilic cavity and an external hydrophilic side. In aqueous solution, the βCD cavity is occupied by water molecules, which are energetically unfavored and therefore can be readily substituted by appropriate "guest molecules" that are less polar than water. The inclusion complex consists of an electrostatic interaction between the torus-like macro-ring of βCD (host) and the active agent (guest) that fits into the lipophilic cavity of the biopolimeric matrix (12).

Aims of the present work were to test the suitability of the triazole fungicide tebuconazole (TBC) to be included in βCD, to determine the βCD−TBC complex composition by magnetic resonance spectroscopy, and to evaluate the fungicidal efficacy of the βCD−TBC complex applied by seed coating in comparison with a commercial formulation of tebuconazole.

Here we describe the methodology for the inclusion of technical TBC with βCD and report on the efficacy of the complex for controlling crown and foot rot on durum wheat caused by *F. culmorum.*

MATERIALS AND METHODS

Materials. β-Cyclodextrin (CAVAMAX7 PHARMA) was obtained from Wacker Chemie Italia (Peschiera Borromeo, Italy) and was used as received. Racemic tebuconazole [(R,S)-(1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*,1,2,4-triazol-1-ylmethyl)pentan-3-ol] was used in 98% purity (NMR). It was extracted with a mixture of water−dichloromethane from a powder formulate (Folicur WG, Bayer CropScience Italia, Milano, Italy) containing 25% of active ingredient.

NMR Measurements. ^{1}H NMR and ^{13}C NMR spectra of the βCD−TBC complex (in D_2O) and ^{1}H NMR of TBC (in $CDCl_3$) were recorded with a Varian spectrometer Mercury Plus (Varian Inc., Palo Alto, CA) at 399.93 and 100.57 MHz, respectively. Chemical shifts are given in parts per million (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet), ddd (double of doublet of doublet), or series of m (series of multiplets). [1]H chemical shifts were referred to H_2O and $CHCl_3$ signals, whereas ^{13}C chemical shifts were referred to external reference (trimethylsilylpropionic acid, sodium salt). ^{1}H NMR and ^{13}C NMR spectra were recorded with a 5 mm tube without degasing.

The release of the TBC from the host was detected by ^{1}H NMR at 22 and 45 °C during the time, respectively.

Preparation of a βCD−TBC Inclusion Complex. Two equivalents (6.50 mmol) of βCD, analytical grade, was dissolved in 160 mL of degassed distilled water under inert atmosphere at 70 °C for 1 h. One equivalent (3.25 mmol) of TBC was added by a spatula, and the reaction mixture was heated at 70 °C under stirring for 18 h. The solution became opalescent and turned milky during the time. A light precipitate was filtered off the solution at room temperature, water was evaporated under vacuum, and a white solid (inclusion complex) was recovered from the solution (90% yield).

Characterization of TBC and the βCD−TBC Inclusion Complex by ^{1}H NMR. *Tebuconazole:* ^{1}H NMR ($CDCl_3$, δ) 1.18 (s, 6H, *t*-Bu), 1.64−1.87 (series of m, 3H, −CH_2), 2.43 (ddd, J = 4.4, 10.8, 11.6 Hz, 1H, −CH_2), 4.34 (s, 2H, −CH_2 benzylic), 6.94 (dd, J = 2.4, 8.4 Hz, 2H, aromatics), 7.18 (dd, J = 2.4, 8.4 Hz, 2H, aromatics), 7.99 (s, 1H, triazole), 8.20 (s, 1H, triazole).

βCD−TBC Inclusion Complex: ^{1}H NMR (unbuffered D_2O, δ) 1.06 (s, 6H, *t*-Bu), 1.72−1.88 (series of m, 3H, −CH_2), 2.56 (m, 1H, −CH_2 benzylic), 3.55 (t, J = 10.0 Hz, 7H, βCD-H$_4$), 3.61 (dd, J = 3.6, 10.0 Hz, 7H, βCD-H$_2$), 3.77 (m, 7H, βCD-H$_5$), 3.83 (s, 14H, βCD-H$_6$), 3.88 (t, J = 10.0 Hz, βCD-H$_3$), 4.50 (m, 2H, −CH_2N), 5.03 (dd, J = 3.6, 10.0 Hz, 7H, βCD-H$_1$), 7.03 (d, J = 8.4 Hz, 1H, aromatic), 7.05 (d,

J = 8.4 Hz, 1H, aromatic), 7.23 (dd, J = 1.6, 8.4 Hz, 2H, aromatic), 8.13 (s, 1H, triazole), 8.56 (s, $^{1}/_2$H, triazole), 8.57 (s, $^{1}/_2$H, triazole).

Greenhouse and Field Experiments. A monosporic isolate of *F. culmorum* (R963, kindly provided by Dr. Christian Barreau, Unité Biologie et Technologie Après Récolte INRA-UBTAR, Villenave d'Ornon, France) was cultured on potato dextrose agar (PDA; Difco, Detroit, MI) at 25 °C with a photoperiod 12 h for 5 days. Plugs (1.2 cm diameter) of PDA colonized by the fungus were cut with a sterile cork borer and used as inoculum. In the greenhouse experiments, one plug was placed in the center of a plastic sowing pot (4.5 cm in diameter, 55 mL capacity), containing sterilized (121 °C for 60 min on two successive days) potting mix (Humin-Substrat N17, Neuhaus, Germany). One seed of durum wheat (*Triticum durum* cv. Prometeo, Istituto Sperimentale per la Cerealicoltura, Roma, Italy) was placed onto each PDA plug and covered by sterilized soil.

The following seed coating treatments were compared: Folicur WG [TBC, 25% active ingredient (ai)], 120 μg g^{-1} of seed; TBC complexed in βCD, 361 μg g^{-1} seed, corresponding to 30 μg of TBC g^{-1} of seed; TBC complexed in βCD, 361 μg g^{-1} seed + carboxymethylcellulose (Sigma, St. Louis, MO) as sticker, 15 mg mL^{-1}; βCD, 361 μg g^{-1} of seed.

For each treatment, 10 (first experiment) or 4 (second experiment) replicates (10 seeds for each replicate) were used. Pots were watered daily, and the average temperature was 25 °C (minimum, 15 °C; maximum, 35 °C).

Seedling emergence was checked every 7 days during 5 weeks. After the last survey, plant height and disease severity were evaluated. The severity of disease was calculated using the McKinney index (13), which expresses the percentage of the maximum severity of disease (i.e., 100) according to the formula $I = [\Sigma (cf)/nN] \times 100$, where c = disease class, f = frequency, n = number of observations, and N = the highest value of an empirical scale adopted, estimating the severity of symptoms on the stem. Five classes were set: class 0 = healthy stem; class 1 = mild browning on the stem; class 2 = browning on one-half of the stem; class 3 = complete browning of the stem; class 4 = plant death after emergence or plant not emerged in comparison with the emergence of the uninoculated control treatment.

In the field experiment, treatments, type of inoculum, and the host plant were identical to those adopted in the greenhouse experiments. Each treatment was replicated three times. For each replicate, 40 seeds were placed on the colonized PDA plugs (1 seed per plug) disposed along a 1 m long furrow (10-cm deep) and covered with sieved natural sandy-loam soil. Seedling emergence was checked after 1 month, and just before harvesting (i.e., 5 months after sowing), the number of heads was counted. The severity of the disease was calculated after harvesting by using the McKinney index. Four classes were set: class 0 = healthy stem; class 1 = mild browning on the first internode; class 2 = first internode entirely discolored and mild browning on the second internode; class 3 = first and second internode entirely discolored. The yield and the weight of 1000 seeds were determined for each treatment.

Statistical Analysis. Data were analyzed using the analysis of variance (ANOVA) for all three experiments separately (two in greenhouse and one under the field conditions).

For emergence and disease severity variables, ANOVA was conducted after transformation of the original data (expressed as percentage) using the $arcsin\sqrt{x}$ function, where x is the relative proportion. This transformation is appropriate to percentage and useful when original data do not fall between 30 and 70% (14). For each experiment and for each variable the means separation was done by calculating the least significant differences (LSD) for a P level of 0.05. For emergence and disease severity variables, the LSD and the mean ranking were calculated using the $arcsin\sqrt{x}$ transformed values; however, to report means in the tables, percentage values were used (14). The correlation among variables (Pearson r) and the associated significance levels (P) were calculated using JMP ver. 3.1.5 software (15).

RESULTS

NMR Properties of the TBC−βCD Inclusion Complex and Determination of the Ratio Guest/Host. NMR spectroscopy, which proved to be very useful in the study of inclusion

482 *J. Agric. Food Chem.,* Vol. 54, No. 2, 2006

Balmas et al.

Table 1. ^{1}H and ^{13}C NMR Shifts (Parts per Million) of β-Cyclodextrin (βCD) and β-Cyclodextrin–Tebuconazole (βCD–TBC) Complex in D_2O and the Corresponding Complexation Shifts ($\Delta\delta = \delta$ Complex − δ Free)

proton	βCD	βCD–TBC	$\Delta\delta^a$	proton	βCD	βCD–TBC	$\Delta\delta^a$
H_1	5.049	5.032	−0.017	C_1	102.51	102.61	0.10
H_2	3.629	3.604	−0.025	C_2	72.70	72.70	
H_3	3.944	3.885	−0.059	C_3	73.71	73.84	0.13
H_4	3.564	3.552	−0.012	C_4	81.76	81.79	0.03
H_5	3.841	3.773	−0.068	C_5	72.43	72.52	0.09
H_6	3.857	3.835	−0.022	C_6	60.89	60.82	−0.07

^{a} $\Delta\delta$ represents the chemical shift differences (ppm) between the two states. Negative values indicate shift to high field.

complexes, was used to probe the intermolecular interactions between guest and host. The chemical shifts of H_3 and H_5 βCD protons, which point out to the lipophilic cavity, are useful probes to observe the formation of inclusion complexes and to evaluate the modification structural level of βCD. Induced changes of the βCD environment caused by the presence of the guest were also reflected in the ^{13}C NMR spectra.

Reasonable water solubility (>3 g L^{−1}) of the βCD−TBC complex at 25 °C gave feasible ^{1}H and ^{13}C NMR experiments in D_2O, thus providing further experimental evidence of the complex formation because free TBC is not water-soluble (30 mg L^{−1} at 20 °C). Moreover, no frequency variation of H_3 and H_5 βCD protons was observed when a ^{1}H NMR spectra of a physical mixture containing βCD−TBC in 1:1, 2:1, and 2.8:1 ratios, respectively, was recorded in D_2O. In that experiment, TBC signals are not clearly detectable and in any case do not represent the real stoichiometry introduced.

Significant high-field shift for H_3 and H_5 βCD proton resonance in the βCD−TBC complex with respect to the free βCD have been measured (**Table 1**). Therefore, the value of the chemical shift changes ($\Delta\delta$) observed reflects the degree of intermolecular proximity and provides information on the strength of guest−host association. The chemical shifts of H_6 are virtually unchanged as are those of H_1, H_2, and H_4 protons located on the external surface of βCD. The chemical shift changes of the βCD carbon atoms caused by interaction with the guest are small, although significant downfield changes have been observed for C_1, C_3, and C_5, respectively.

In the NMR proton of the βCD−TBC complex recorded in D_2O it is interesting to note two singlet signals in 1:1 ratio attributed to one triazole proton located at 8.56 and 8.57 ppm, respectively. Because no tautomerism would be present at the triazole ring, the duplicated signal observed at 8.56 and 8.57 ppm might be attributed to the presence of two diastereomeric

βCD−TBC complexes. No chemical shift change of the signal pattern was observed when the sample was heated at 45 °C for 2 h.

A 2.8 ratio of βCD−TBC was calculated by integration of ^{1}H NMR βCD−TBC signals of the solid (90% yield) recorded in D_2O. Preparation, starting from an equimolar ratio of βCD and TBC, gave still a βCD−TBC complex in 2.8 ratio but a lower yield (<60%) was calculated when compared to the starting 2:1 stoichiometry of host to guest.

Controlled release of TBC from the host measured in D_2O by chemical shift of the diagnostic proton H_3 and H_5 of βCD confirmed a good stability of the complex at the solid state after storage at 22 °C for 14 months.

The release of the TBC in aqueous solution was detected by ^{1}H NMR in D_2O at 25 and 45 °C during the time, respectively. TBC releases from the βCD after 3 days at 25 °C and after 2 h at 45 °C. The release of the guest was detected on the basis of two different pieces of evidence: first, the signal of H_3 and H_5 βCD protons shifts until it reaches the chemical shift present in free βCD; second, a decrease of the ratio of TBC to βCD was calculated by the releasing time.

Control of Foot and Crown Rot on Durum Wheat by Different Tebuconazole Formulates. In the greenhouse experiments, the seed dressing source of variation was significant for all of the variables considered (**Table 2**). Plant emergence and plant height of durum wheat sown in artificially infested substrate were significantly improved by the seed treatments with TBC, regardless of the formulation adopted (**Table 3**). The use of the βCD−TBC complex, with or without the addition of CMC, contained disease severity to 5.6−9.5% even in the presence of a high disease pressure (73−83% in the inoculated control), although this effect was significantly different from that obtained following the application of a commercial formulation of TBC only in the first experiment (**Table 3**). Seed coating with βCD alone had limited but significant effect on plant height and disease severity caused by *F. culmorum* (**Table 3**). Results obtained in the two greenhouse experiments were consistent ($r = 0.887−0.994$; $P < 0.05$; $n = 6$; **Table 3**).

In the field experiment, no significant differences between treatments were observed when the effect on seedling emergence or the weight of 1000 seeds was considered (**Tables 4** and **5**). On the contrary, seed dressing had a significant impact on the extent of disease severity (**Table 4**), despite the fact that treatments with TBC were not different from each other (**Table 5**). It should be also noted that, compared to the greenhouse experiments, treatments with TBC were less effective in limiting disease severity (**Tables 3** and **5**). Among the three seed dressing treatments tested, only the βCD−TBC complex allowed a yield that was not different from that produced by the uninoculated control (**Table 3**). No significant differences in the number of

Table 2. Results of the Analysis of Variance (ANOVA) for the Variables Emergence, Plant Height, and Disease Severity in Durum Wheat (Cv. Prometeo)^a

		first greenhouse expt Dec 1, 2003–Jan 7, 2004				second greenhouse expt Dec 7, 2004–Jan 13, 2005		
		SS				SS		
source of variation	df	emergence^b	plant height	disease severity^b	df	emergence^b	plant height	disease severity^b
model	14	7.33	228.30	9.00	8	3.60	540.35	4.37
seed dressing	5	6.89***	192.14***	8.59***	5	3.47***	492.83***	4.07***
replicates	9	0.44	32.77	0.41*	3	0.13	47.52	0.30*
error	45^c	1.54	170.34	0.91	15	0.66	126.16	0.45

^{a} The trial was conducted in greenhouse by sowing in a substrate artificially infested with *F. culmorum* after six different seed dressing treatments. ANOVA results are presented separately for the two greenhouse experiments. df = degree of freeedom; SS, sum of squares. Significance levels: *, $P < 0.05$; ***, $P < 0.0001$. ^{b} For these variables, ANOVA was performed by using arcsin$\sqrt{x}$ transformation of the original variable (%). ^{c} For plant height df = 44.

Control of Foot and Crown Rot of Durum Wheat

J. Agric. Food Chem., Vol. 54, No. 2, 2006 **483**

Table 3. Emergence, Plant Height, and Severity of Foot and Crown Rot Caused by *F. culmorum* in Durum Wheat (Cv. Prometeo) Sown in Artificially Infested Substrate after Seed Dressing with Different Formulations of Tebuconazole and β-Cyclodextrin[a]

seed dressing	dosage (μg of ai g^{-1} of seed)	inoculum	emergence[b] (%) I	emergence[b] (%) II	plant height (cm) I	plant height (cm) II	disease severity[b] (%) I	disease severity[b] (%) II
	–	–	76.0b	97.5a	20.2a	30.9a	0.3e	0.0c
	–	+	35.0c	32.5b	15.1c	19.4b	73.0a	82.8a
TBC (Folicur WG, 25% ai)	30	+	95.0a	97.5a	19.9ab	28.2a	13.3c	13.1b
TBC complexed in βCD	30	+	94.0a	95.0a	20.2a	29.5a	5.6d	9.5b
TBC complexed in βCD + CMC	30	+	97.0a	100.0a	20.3a	28.3a	7.4cd	8.8b
BCD	–	+	47.0c	52.5b	18.2b	20.2b	59.7b	62.2a
mean			74.0	79.2	19.1	26.1	26.6	29.4
correlation[c]			$r = 0.936$**		$r = 0.887$*		$r = 0.994$***	

[a] Means are shown for the variables in the first (I) and second (II) greenhouse experiments. For each variable, the correlation (Pearson *r*) among the means of the treatments in the two experiments is also presented. Values in the same column followed by the same letter do not differ statistically by using LSD for *P* = 0.05. TBC, tebuconazole; βCD, β-cyclodextrin; CMC, carboxymethylcellulose. [b] The variables emergence and disease severity are presented as percentage (%) values; however, the separation of the means was performed using the arcsin$\sqrt{x}$ transformed variables. [c] Significance levels: *, *P* < 0.05; **, *P* < 0.01; ***, *P* = 0.0001. For the variables emergence and disease severity correlation was calculated using the arcsin$\sqrt{x}$ transformed variables.

Table 4. Results of the Analysis of Variance (ANOVA) for the Variables Emergence, Disease Severity, Yield, Number of Heads per Linear Meter, and Weight of 1000 Kernels in Durum Wheat (Cv. Prometeo)[a]

source of variation	df	SS emergence[b]	SS disease severity[b]	SS yield (g)	SS no. of heads (m^{-1})	SS wt of 1000 kernels
model	7	0.10	0.29	15616.74	2566.17	41.88
seed dressing	5	0.09	0.26***	8742.29*	1467.17*	37.95
replicates	2	0.02	0.00	6874.45**	1099.00*	1.97
error	10	0.13	0.02	1964.82	474.33	42.13

[a] The trial was conducted under field condition sowing in soil artificially infested with *F. culmorum* after six different seed dressing treatments. df, degree of freedom; SS, sum of squares; TBC, tebuconazole; βCD, β-cyclodextrin; CMC, carboxymethylcellulose. Significance levels: ***, *P* < 0.0001; **, *P* < 0.001; *, *P* < 0.01. Values in the same column followed by the same letter do not differ statistically by using LSD for *P* = 0.05. [b] For these variables, ANOVA was performed using arcsin(*x*) transformation of the original variable (%).

Table 5. Emergence, Severity of Foot and Crown Rot Caused by *F. culmorum*, Total Yield, Number of Heads Harvested per Linear Meter, and Weight of 1000 Seeds Produced by Durum Wheat (Cv. Prometeo) Sown in Artificially Infested Soil after Seed Dressing with Different Formulations of Tebuconazole and β-Cyclodextrin (Field Experiment, February 24–June 6, 2004) (Means Shown for Each Variable)[a]

seed dressing	dosage (μg of ai g^{-1} of seed)	inoculum	emergence[b] (%)	disease severity[b] (%)	yield (g)	no. of heads (m^{-1})	wt of 1000 kernels
	–	–	73.3	13.7c	137.3a	80.3a	37.7
	–	+	60.0	45.0a	77.1c	59.7bc	35.4
TBC (Folicur WG, 25% ai)	30	+	69.2	37.0b	96.1bc	65.3b	36.4
TBC complexed in βCD	30	+	69.2	36.1b	115.7ab	66.3b	39.0
TBC complexed in βCD + CMC	30	+	59.2	32.3b	110.1bc	65.3b	38.5
βCD	–	+	55.0	49.1a	74.4c	50.0c	35.2
mean			64.3	35.5	101.8	64.5	37.0

[a] Values in the same column followed by the same letter do not differ statistically by using LSD for *P* = 0.05. [b] The variables emergence and disease severity are presented as percentage (%) values; separation of the means was performed using the arcsin$\sqrt{x}$ transformed variables.

heads per linear meter were observed between different formulations of TBC (**Table 5**), although seed dressing affected this parameter significantly (**Table 4**). Differences in yield among treatments were explained by differences in the number of harvested heads per linear meter ($r = 0.926$; *P* < 0.01; *n* = 6).

DISCUSSION

In cereals, the use of seed dressing is a crucial treatment for the control of soilborne diseases. *F. culmorum* is considered to be one of the most damaging soilborne fungi in many cereal crops, especially wheat. This study confirms the aptness of the βCD inclusion complex in agricultural practices and particularly in fungicide seed-coating formulations. The possibility to incorporate fungicides in βCD for the control of plant diseases has already been described (*9–11*), as well as other methods for controlled release of fungicide by using nanoparticles (*16*). However, not all chemical compounds can be included in βCD,

because particular electrostatic and steric features are requested. Here we describe an inexpensive method to prepare βCD−TBC complex in water in virtually any quantitative yield. The complete analysis of the βCD−TBC complex was investigated by spectroscopic magnetic resonance in D$_2$O without further treatment, to determine the real complex composition. TBC proved to be an efficient guest for βCD, which forms a stable complex where one molecule of the fungicide seems to be included between two molecules of βCD.

Significant chemical shift of the H$_3$ and H$_5$ of βCD supports the evidence of a true complex, whereas the chemical shift changes of the βCD carbon atoms caused by interaction with the guest are small, although significant downfield changes have been observed for C$_1$, C$_3$, and C$_5$, respectively.

Given the very poor water solubility of TBC (30 mg L^{-1} at 20 °C), complexation in βCD provides increased aqueous solubility to the fungicide and therefore better bioavailability.

484 *J. Agric. Food Chem.,* Vol. 54, No. 2, 2006

Balmas et al.

Our studies indicate that the βCD−TBC complex is stable for months when stored at room temperature at the solid state and that it is also reasonably stable in water (3 days at 25 °C and 2 h at 45 °C).

Due to the presence of a stereogenic center in the TBC molecule, the formation of two diastereomeric βCD−TBC complexes should be taken into account: our results support the hypothesis that the two singlet signals of one triazole proton observed in the NMR proton spectrum may be assigned to two diastereomeric βCD−TBC complexes.

Results obtained under greenhouse conditions show that there is no significant difference between the use of a commercial formulation of TBC and the application of the same chemical compound included in βCD for coating seeds of durum wheat for the control of crown and foot rot caused by *F. culmorum* during the first vegetative stage. Results from the field experiment also suggest that yield may be improved by the βCD−TBC complex, although a multienvironmental trial is needed to take into account a more realistic agronomic scenario. These preliminary results prove that by the use of the βCD−TBC complex it is possible to obtain the release of the active chemical agent without compromising its effectiveness.

The high stability of the complex at low temperature possibly allows protection of the seedlings of winter wheat for a longer period against soilborne pathogenic fungi: the release of the fungicide is enhanced at increasing soil temperatures, which also correspond to an increased activity of the pathogens. In the field experiment, no differences were observed between the emergence of the controls (healthy, disease, and βCD control) and that of the fungicide-coated treatments: this may be explained by the fact that the average temperature was quite low (9 °C in March 2004) during the first vegetative stage of the crop, and the pathogen may not have influenced seedling emergence.

Future experiments will be carried out to evaluate the effectiveness of seed coating with the βCD−TBC complex in reducing the incidence of foliar diseases of durum wheat, such as *Blumeria graminis* (DC.) Speer, *Mycosphaerella* sp., and *Pyrenophora* sp. These experiments should elucidate whether a slow release of the fungicide may allow protection of the plant to be extended during the growth cycle.

It has not escaped our notice that, due to the great importance of triazole fungicides in plant protection strategies, inclusion of these compounds in βCD should be regarded as a most sustainable and economical use, especially when the fungicide is applied in enantiopure form (*17*−*19*). This would allow the patent term to be extended by strongly limiting toxicological requirements needed for registering a new formulate.

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