# Triticonazole Distribution in Dressed Corn Caryopsis and Seedlings

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A corn seed dressing with the fungicide triticonazole at 760 nmol/seed prevents head smut disease. In resting seeds, the dressing treatment was followed by the penetration of 19% of the product, 9% inside the tegument and 7% inside the pedicel. In growing seedlings, the inner content increased in the storage organs (endosperm + scutellum) as well as in the growing organs. The partition lipophilic phase/water certainly explains the high apparent fungicide concentration progressively reached inside endosperm and scutellum. However, no important transfer of fungicide from these organs to the growing parts seems to occur. It appears therefore that the fungicide transfer from the coating to the roots mostly occurs through dissolution of the product in the surrounding soil water and through root absorption. The efficient fungicide concentration inside the meristem is likely to be obtained during the early stages of development.

**Keywords:** *Zea mays L.; fungicide; seed coating; bioavailability*

## INTRODUCTION

Seed dressing has proved its usefulness for several years especially in the case of seedling fungal diseases. The seed dressing technique allows two phases of pesticide absorption to occur inside the seed: the imbibition phase and the period of seedling growth (Hocombe, 1968). The penetrated pesticide amount depends on its concentration in the external water solution (Walles, 1976), and Rubin and Demeter (1986) admitted that the absorption process was due to simple diffusion.

For diseases concerning the germinating seed directly, contact fungicides preventing spore germination in the soil surrounding the seeds have shown their efficiency as soon as the imbibition phase begins (Schiffers et al., 1988). Furthermore, seed dressing has now been shown to give interesting effects in the case of fungal diseases resulting from a much more complex cycle. This is the case of head smut disease, in corn, which is due to *Sphacelotheca reiliana*. This disease induces male flower necrosis in developed plants, but the fungal infection was shown to occur very early, during or immediately after germination of the seed (Maytac, 1985; Vergnet and Elbe, 1989). In this case, sterol biosynthesis inhibitors were shown to be effective when used with the same seed dressing method. For instance, triticonazole at 760 nmol/seed gave a good protection against corn head smut diseases.

However, with this type of compound, fungicidal action was supposed to occur exclusively inside the germinating seed or growing seedling. The purpose of this research was to show the triticonazole distribution inside the different parts of the seedling, as a function of time, and to try to establish the main ways of transfer of the fungicide from the seed dressing to the seedling organs and especially to show whether a high rate of transfer of the product could occur from the storage tissues to the seedling.

## MATERIALS AND METHODS

**Chemicals and Plant Materials.** Triticonazole ((1*RS*)-(*E*)-5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentan-1-ol) and [$^{14}$C]triticonazole ([$^{14}$C]phenyl, specific activity = 1.18 GBq mol$^{-1}$) were provided by Rhône-Poulenc Agro (Lyon, France). The corn seeds (*Zea mays* L. cv. Anjou 285 and cv. Furio) were provided by Biocem (Limagrain, Angers, France). The dressed corn seeds cv. Anjou 285 (triticonazole = 66.7 g q$^{-1}$, 100 000 dpm [$^{14}$C]triticonazole/seed) were prepared by Rhône-Poulenc Agro (Lyon, France).

**Corn Seedling Culture.** *Study of Triticonazole Transfer from an Aqueous Solution to the Seedlings.* Corn seeds were germinated in Petri dishes for 4 days. At this stage, the seedlings were fully immersed in a 10 $\mu$M triticonazole solution (15 seedlings for 300 mL of solution) as described by Zimmerlin and Durst (1990). After 2, 4, or 6 days, the seedlings were washed with water for 5 s and dissected.

*Study of Triticonazole Transfer from the Seed Dressing to the Inner Parts.* Dressed corn seeds were germinated in Petri dishes (10 seeds in 10 mL of water) at 30 °C in the dark. After 2, 4, or 6 days of growth, the seedlings were taken out and washed with water for 5 s. Afterward, the seed tegument was cautiously wiped with a filter paper saturated with ethanol followed with water. The triticonazole amount obtained in that way was measured, and the seedlings were dissected in order to separate pedicel, tegument, endosperm, scutellum, and embryo (for the first stage) and roots, mesocotyle, and other aerial parts (coleoptile + leaves) at later stages.

**Radioactivity Measurements and Autoradiographies.** All dissected seedling organs were weighed, crushed with a mortar, and extracted with ethanol/water (1:1 v/v). The radioactivity detection was carried out as described previously

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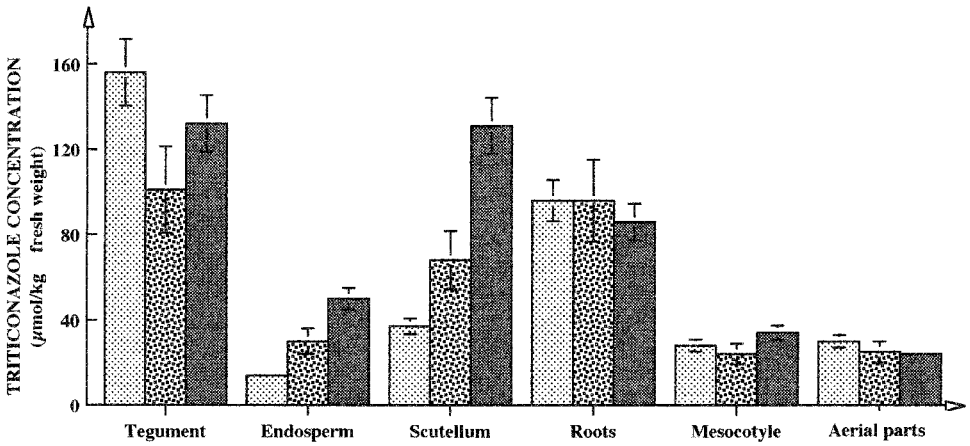


**Figure 1.** Triticonazole distribution inside corn seedlings (cv. Anjou 285) immersed in a 10 $\mu$M triticonazole aqueous solution: left bars, 2 days; middle bars, 4 days; right bars, 6 days. Error bars: $\pm\sigma$ (3 replicates).

**Table 1. Six-Day-Old Corn Seedling Composition (%)**

| | tegument | endosperm | scutellum | roots | mesocotyle | aerial parts |
|---|---|---|---|---|---|---|
| water | 57.2 | 51.2 | 62.9 | 88.7 | 92 | 90.6 |
| extractable lipids | 2.9 | 1 | 9.9 | 0.3 | 0.2 | 0.4 |
| other constituents | 39.9 | 47.8 | 27.2 | 11 | 7.8 | 9 |

by Raveton et al. (1997). After centrifugation (5000*g*, 10 min), the supernatant was collected and deposited on TLC silica gel plates (Merck, 60F254). The products were separated by petroleum ether (35−60 °C)/ethyl acetate/methanol (5:5:2 v/v/v). Autoradiographies of TLC plates were performed with Kodak films (DEF 5).

**Triticonazole Flux via Endosperm.** To study this type of transfer, we perforated the tegument and the endosperm of dry untreated seeds. In the hole created in the endosperm (diameter, 1 mm; depth, 2 mm) with the help of a microdrilling machine, 10 $\mu$L of an ethanolic solution of triticonazole was injected (6 nmol, 200 000 dpm/caryopsis). The seeds remained at room temperature for 12 h for the ethanol to evaporate. The seeds were then germinated in Petri dishes. The seedlings were dissected after 2 days. The aqueous solution in the Petri dishes was used as the sample to verify the presence or absence of radioactivity.

**Lipid Extraction.** Fresh material (5 g) was extracted with acetone/water (4:1 v/v; 3 × 50 mL) and the solid fraction filtered.The filtrate was extracted with petroleum distillate (3 × 10 mL). The lipophilic phase was concentrated to dryness under vacuum and weighed.

## RESULTS AND DISCUSSION

**Untreated Corn Seeds Immersed in a 10 $\mu$M Aqueous Triticonazole Solution.** Two corn varieties were concurrently studied: Anjou 285 and Furio. In each, the triticonazole structure remained unmodified throughout the experiment; no metabolites were detected in contrast with what occurs in wheat seedlings (Quérou, 1997).

The growth of the seedlings was not affected by triticonazole, and the fungicide distribution appeared the same for both corn cultures. Figure 1 illustrates the distribution in Anjou 285. It represents the apparent concentration of triticonazole in each seed organ (seed coat, endosperm, scutellum, and newly formed organs: roots, mesocotyle, aerial parts). This concentration was measured 2, 4, and 6 days after the beginning of the experiment which corresponded to the seed imbibition period. Figure 1 shows that the apparent concentrations measured as $\mu$mol of triticonazole/kg of fresh weight were always very high as compared to the 10 $\mu$M concentration in the external water. For instance, after 6 days, the apparent concentration was 130 $\mu$mol kg$^{-1}$ FW for the tegument and scutellum, 85 for the roots, 50 for the endosperm, and 30−35 for the mesocotyle and aerial parts.

As previously demonstrated (Albertin et al., 1994; Raveton et al., 1997) so high a concentration can be explained: (1) through a passive diffusion process, triticonazole concentration in the inner cell water increased to reach a 10 $\mu$M equilibrium value; (2) at the same time, triticonazole was concentrated in the lipophilic parts of the cells, mostly with the extractable lipids present in each compartment of the seedling (Table 1).

The high values of the apparent concentration obtained in the case of the tegument and scutellum are in agreement with their high content in extractable lipids. In the case of roots, a high apparent concentration was also obtained, the origin of which was necessarily different.

The apparent triticonazole concentration in tegument, roots, and other young parts was almost constant from days 2 to 6. This suggests that the exchange between the medium and these organs was intense enough to allow the partition diffusion equilibrium to be reached before day 2. This can be understood in the case of tegument, which has a high exchange surface with the medium. In the case of newly formed parts, this suggests that triticonazole mainly penetrated into these poorly protected growing organs directly from the medium.

In contrast, the apparent concentration in the endosperm and in the scutellum increased slowly from day 2 to 6. This demonstrates that the flux of exchange from the scutellum to the inner tissues was relatively slow and that the true diffusion partition equilibrium was probably not reached in these tissues even at the end of the experiment.

When measuring the amount of triticonazole inside each organ of one seed, we can see that 21% of the penetrated fungicide was distributed inside the endosperm at the end of the experiment, 20% inside the scutellum, 27% in the roots, 18% in the tegument + pedicel, and 14% in the aerial parts. The rate of

**Table 2. Triticonazole Concentration and Content in Various Corn Organs of Dressed Resting Caryopsis**

| | pedicel | tegument | endosperm | scutellum | embryo |
|---|---|---|---|---|---|
| triticonazole concn ($\mu$mol kg$^{-1}$ FW) | 6370 ± 380 | 1680 ± 180 | 40 ± 5 | 135 ± 110 | 350 ± 220 |
| triticonazole/seed (nmol) | 45 ± 3 | 55 ± 6 | 12 ± 1.5 | 3.5 ± 2.9 | 1.6 ± 1 |

triticonazole exchange between the medium and seed was 22 nmol/day/seed for the first 2 days, 6.6 nmol/day/seed for days 2−4, and 3 nmol/day/seed for days 4−6.

**Triticonazole Distribution from an Artificial Deposit Inside the Endosperm.** The preceding experiment suggested that perhaps only a direct transfer of fungicide from the medium to the growing parts was occurring, since the transfer from the storage compartments to the growing parts was supposed to be negligible. It was therefore interesting to show whether a transfer from the endosperm to the growing parts could exist or not. For this purpose, we demonstrated that a hole (able to be filled with a fungicide solution) could be made inside the endosperm behind the scutellum without changing the germinating or growing properties of the seed.

Two days after injection of the fungicide inside the endosperm, 75% of the product remained inside the endosperm. Triticonazole moved limitedly into the scutellum (6.7%) and hardly to the newly formed parts (0.8%). Less than 6% of the product was measured in the external medium showing that the dissolution of a small part of triticonazole inside the medium could not be avoided. This experiment strongly suggests that the transfer of product from the endosperm to the young growing parts was unlikely to occur. Under our conditions, the source to sink phloem movement from the seed to the growing parts does not seem to transfer significant amounts of triticonazole.

These results show that the major part of the product found in the growing parts (>20%) in the first experiment comes directly from the aqueous medium.

**Triticonazole Distribution from Seed Dressing.** Two steps of triticonazole distribution had to be considered initially: just after the seed dressing and when water was applied to the seed for germination and growth.

*Distribution Inside the Resting Seeds.* The amount of triticonazole associated with dry dressed seeds was 760 nmol/seed, corresponding to 2.7 mmol kg$^{-1}$ seeds. After superficial washing of the seed dressing as explained in Materials and Methods, a maximum of 19% of the whole fungicide amount remained in the seed. Most of it was present in the tegument (9%) and the pedicel (7%). Inside this external layer (tegument + pedicel) the apparent concentration reached 2.5 mmol kg$^{-1}$ fresh weight. Only 3% of the 19% present in washed seeds was found in the inner tissues of the corn caryopsis (2% in the endosperm, 0.6% in the scutellum, and 0.3% in the embryo).

The average concentrations inside the scutellum and embryo were relatively high but with a very large standard error. In fact, the triticonazole concentration in the embryo varied between 26 and 600 $\mu$mol kg$^{-1}$ FW (Table 2). This suggests that the tegument impermeability above the embryo might greatly change from one seed to the others.

*Triticonazole Distribution during the Germination of Dressed Seeds.* Figure 2 shows the kinetics of the triticonazole inner content in germinating dressed seeds for 6 days. The fungicide content was increasing for two main reasons: (1) the concentration increased markedly

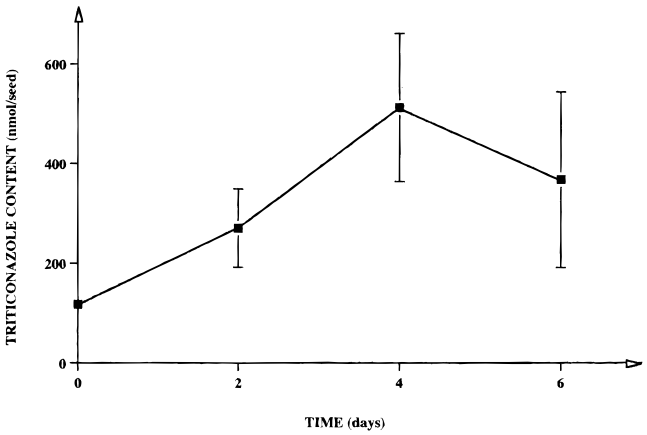


**Figure 2.** Triticonazole inner content in dressed germinating corn seeds (the superficial seed dressing was washed before measurements). Error bars: $\pm\sigma$ (3 replicates).

**Table 3. Triticonazole Concentration ($\mu$mol kg$^{-1}$ FW) in Different Parts of Dressed Germinating Caryopsis and as a Function of Time[a]**

| | time (days) | DS | WDS + pedicel | WDS − pedicel |
|---|---|---|---|---|
| pedicel | 2 | 11920 | 3420 | |
| | 4 | 29620 | 5360 | |
| | 6 | 10020 | 2480 | |
| tegument | 2 | 1530 | 440 | 470 |
| | 4 | 1770 | 350 | 200 |
| | 6 | 1740 | 270 | 270 |
| endosperm | 2 | 200 | 46 | 25 |
| | 4 | 270 | 70 | 48 |
| | 6 | 350 | 53 | 42 |
| scutellum | 2 | 290 | 100 | 63 |
| | 4 | 450 | 170 | 100 |
| | 6 | 810 | 110 | 97 |
| roots | 2 | 280 | 61 | 28 |
| | 4 | 210 | 55 | 33 |
| | 6 | 240 | 46 | 44 |
| mesocotyle | 2 | | | 18 |
| | 4 | 60 | 15 | 11 |
| | 6 | 50 | 16 | 8 |
| aerial parts | 2 | 120 | 43 | 23 |
| | 4 | 50 | 13 | 4 |
| | 6 | 20 | 3 | 3 |

[a] DS, dressed seeds; WDS + pedicel, washed dressed seeds with pedicel; WDS − pedicel, washed dressed seeds without pedicel.

in the pedicel, endosperm, and scutellum; (2) the newly formed organs were growing. In these new organs, triticonazole was mostly found in the roots which maintained a constant concentration (Table 3). Under these conditions, the triticonazole concentration in the water surrounding the seeds was 60 $\mu$M, most of the seed dressing being washed off and sedimented in the dishes. In this experiment, it is noteworthy that the tegument concentration remained constant and at the same level as in the resting seed (Table 2), in strong contrast to the pedicel, in which triticonazole concentration approximately doubled during the imbibition stage.

The values indicated in Table 4 show that, after 6 days, the content in the protective tissues (tegument + pedicel) was almost the same as that in the storage tissues (endosperm + scutellum): 150 nmol/organ. The content of the newly formed organs was 2.5 times lower.

**Table 4. Changes in Triticonazole Content (nmol/organ) in Each Organ of Germinating Seeds[a]**

| | time (days) | DS | WDS + pedicel | WDS − pedicel |
|---|---|---|---|---|
| pedicel | 2 | 126 | 42 | |
| | 4 | 288 | 74 | |
| | 6 | 98 | 34 | |
| tegument | 2 | 52 | 15 | 18 |
| | 4 | 63 | 11 | 8 |
| | 6 | 54 | 12 | 11 |
| endosperm | 2 | 68 | 16 | 9 |
| | 4 | 96 | 21 | 16 |
| | 6 | 106 | 15 | 12 |
| scutellum | 2 | 16 | 6 | 4 |
| | 4 | 33 | 11 | 7 |
| | 6 | 62 | 9 | 7 |
| roots | 2 | 6 | 1 | 1 |
| | 4 | 25 | 6 | 6 |
| | 6 | 39 | 13 | 9.5 |
| mesocotyle | 2 | | | 0.2 |
| | 4 | 3 | 0.5 | 0.7 |
| | 6 | 4 | 1 | 0.6 |
| aerial parts | 2 | 1.6 | 0.5 | 0.2 |
| | 4 | 3 | 0.7 | 0.4 |
| | 6 | 3.5 | 0.5 | 0.5 |

[a] DS, dressed seeds; WDS + pedicel, washed dressed seeds with pedicel; WDS − pedicel, washed dressed seeds without pedicel.

**Table 5. Daily Flux of Triticonazole (nmol/day/seed) into Germinating Dressed Seeds and Seedlings**

| | 0−2 days | 2−4 days | 4−6 days |
|---|---|---|---|
| endosperm + scutellum | 42 | 23 | 19.3 |
| growing parts | 3.7 | 12 | 7.7 |
| total | 45.7 | 35 | 27 |

In wheat seeds, the triticonazole distribution differs, due to the presence of a particular tissue, the testa, which played the role of a filter during the imbibition phase (Thiebert et al., 1986, 1988). At day 6, the whole triticonazole content of the germinating seed represented 48% of the initial deposit on the seed. The values shown in Table 4 allow the calculation of daily flux of triticonazole into the storage and growing organs (Table 5).

**Triticonazole Distribution in Germinating Dressed Seeds, with or without Pedicel and Washed or Not Washed.** Tables 3 and 4 show the results of these experiments. In dressed seeds, which were washed prior to imbibition, the tegument concentration which was 1680 $\mu$mol kg$^{-1}$ in the dry seed decreased sharply during the first 2 days, reaching 440 $\mu$mol kg$^{-1}$ and decreased slowly afterward. A similar situation was observed for the pedicel. Under these conditions, triticonazole concentration in the surrounding water reached 20 $\mu$M.

The content in the storage tissues (endosperm + scutellum) remained almost constant during the 6 days (26 ± 5 nmol). However, the root content increased during the experiment at a rate which was directly associated with the concentration in the surrounding water (20 $\mu$M: 3 times lower than in the case of dressed seeds).

In washed seeds where the pedicel was suppressed, the tegument content remained the same as in the preceding case. The triticonazole content of the storage organs remained approximately unchanged. The concentration in the surrounding water reached 10 $\mu$M. For the most part, this concentration was obtained by redissolution of the product contained in the tegument. As expected, the root content increased as a function of the water concentration.

Corn seed dressing carried out under our conditions results in the deposit of solid fungicide on the seed of 81% and in the penetration of the residual of 19% mostly in the superficial tissues (tegument and pedicel). The pedicel is a way of especially easy penetration for the product as well as for water. The fungicidal apparent concentration in this organ is always very high. The distribution of triticonazole inside the seed and the seedling is a phenomenon which is mostly determined by two processes: diffusion and partition in lipophilic compounds which can be actual extractable lipids as in the case of scutellum storage lipids or polymeric lipophilic compounds such as lignine or lipophilic proteins (Phillips et al., 1972; Raveton et al., 1997). Triticonazole is a moderate lipophilic compound (log $P$ = 2.5). Its theoretical concentration inside lipids can therefore be supposed to reach a value close to 315 molecules in lipids for 1 in water. In fact, when the diffusion partition equilibrium seemed to be obtained, the highest factor of apparent concentration in the scutellum observed here was 13 parts/g of scutellum fresh weight, for 1 part/mL of water. Taking into account the lipid content of this organ, this could give a partition factor lipid/water of 125 for this fungicide.

The specific interest of the triticonazole dressing of corn seeds is that it could prevent head smut. It is known now that this fungal infection occurs very early, at the very beginning of seedling growth, but that it could also take place later (Teferi et al., 1989).

Two types of effects can be expected from the treatment. First, in the soil surrounding the seed, the fungicide can prevent fungal development and seedling infestation, but this is unlikely in the case of triticonazole which poorly inhibits spore germination of this fungus. Most of the product will remain in this soil and be washed away with heavy rainfall. Through diffusion, the transfer of this product has been shown elsewhere to be very slow in the soil. With root growth, the young growing parts of these organs are likely to leave the protected soil area after 1 week.

Second, inside the seedling, a fungicide, or at least a fungistatic effect, can be expected from triticonazole, inhibiting mycelium growth. This effect probably only concerns the growing parts and not the storage organs. For obtaining this protective effect, a threshold concentration of the fungicide has to be reached, the value of which has not been precisely measured up to now.

However, this work has shown that the embryo content of triticonazole inside the resting seed varies from one seed to the other between 26 and 600 $\mu$mol kg$^{-1}$ fresh weight. We presently have no evidence that the lowest concentration was effective against a head smut disease precocious attack. In the seedlings, the apparent concentration obtained inside the roots (>200 $\mu$M) seems so high that it cannot be suspected to be lower than the critical concentration inhibiting fungal development. What is known about the efficiency of triazoles inhibiting sterol biosynthesis confirms that point.

It can therefore be suggested that the young seedling is powerfully protected against a head smut attack occurring at this step. Thus, the remaining question seems to concern later steps of seedling development, when triticonazole concentration is probably decreasing in the aerial meristematic parts and when main root absorption no longer occurs in the sphere surrounding the seed and the soil containing the fungicide.

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