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Controlled Release of Tebuconazole from a Polymer Matrix Microparticle: Release Kinetics and Length of Efficacy

JAWED ASRAR,* YIWEI DING,§ RITA E. LA MONICA, AND LINDA C. NESS

Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167

Preparation and characterization of microencapsulated tebuconazole, its release kinetics in water, and the bioefficacy of these formulations in controlling wheat rust in spring wheat is described herein. Controlled-release (CR) formulations based on matrix microparticles were prepared by the oil-in-water emulsion process. Polymer-based matrix was prepared from poly(methyl methacrylate) (PMMA) and poly(styrene-*co*-maleic anhydride) (PSMA). Modification of the matrix was achieved by the use of different low molecular weight or polymeric additives. These additives were found to lower the glass transition temperature of the matrix and enhance the release rate of tebuconazole in water, under infinite sink conditions. Release of tebuconazole from matrix microparticles was found to be diffusion controlled. CR formulations were found to be very efficacious in controlling wheat rust. Soil-applied CR formulations prepared from a PMMA or PSMA matrix, modified with poly(vinyl acetate), were as effective in controlling wheat rust (*Puccinia recondita*) as foliar-applied tebuconazole, Raxil, from Bayer AG. Results suggest that CR formulations with a systemic fungicide, such as tebuconazole, applied as either a soil or seed treatment, may provide control of foliar diseases, possibly eliminating or reducing the need for traditional foliar applications.

KEYWORDS: Tebuconazole; controlled release; microparticles

INTRODUCTION

Seed treatment (ST) with agrochemicals has been an effective method to control a variety of insect pests and diseases (*1*). Systemic materials, such as tebuconazole, triticonazole, fludioxonil, silthiofam, imidacloprid, thiamethoxam, and fipronil, provide very good broad spectrum activity and excellent control of diseases and insect pests, particularly in early crop growth stages. These systemic pesticides also have less hazardous toxicological and ecotoxicological profiles than commercially applied broadcast or in-furrow options. The major crops that benefit from the use of ST are cereals, maize, cotton, potatoes, oilseed rape, and sugar beet. Seed treatment actually reduces the amount of active ingredient applied to the environment because treatment is restricted to the target crop. Although several insecticides and fungicides available for ST are systemic in nature, they typically can provide protection from diseases and pests only to the seed and young seedlings. Generally, effectiveness of the ST is lost with time due to environmental degradation, poor uptake efficiency, and/or short half-life of the active ingredient (AI) in the plant tissue. Maintaining effective levels of AI at later stages of plant growth by the use of ST also becomes challenging due to increasing biomass. Most AIs used as a ST are capable of causing some degree of phytotoxicity, which places a limit on the amount of AI that can be applied directly to the seed. Thus, in general, AIs used for ST ideally require reduced phytotoxicity and prolonged pest control.

Tebuconazole is a systemic fungicide that is effective against various smut and bunt diseases of cereals (*2*). A seed treatment formulation of tebuconazole is commercially available from Bayer AG as a flowable suspension with the trade name of Raxil. Tebuconazole, like other triazole fungicides, is phytotoxic. Excessive amounts of tebuconazole can damage seeds and reduce germination. The application rate recommended for ST (2−25 g of AI/100 kg of seed) is very effective against early-season diseases. However, late-season disease control requires a foliar application.

CR formulations based on microencapsulation could provide a method to reduce phytotoxicity and increase length of activity (*3*). Better operator safety and lower application rate could be achieved. Another significant benefit with encapsulation technology is that it is possible to formulate incompatible pesticides. Matrix microparticles prepared from the oil-in-water emulsion process are typically small particles in which an AI or a mixture of different AIs is dispersed throughout a matrix material (*4*). The mathematics of the release of actives from matrix systems have been discussed by several authors (*5*, *6*).

Park et al. (*7*) described different types of matrix microparticles and recognized that the choice of microparticle, system, and matrix must be tailored to the physical and chemical properties and to the mode of action of individual active

* Address correspondence to this author at Advance Polymer Group LLC, 14949 Royalbrook Dr., Chesterfield, MO 63017 [telephone (314) 374-4289; e-mail jasrar2@aol.com].

§ Present address: Crosslink Polymer Research, 950 Bolger Ct., Fenton, MO 63026.

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compounds. In particular, Park focused on the production of microparticles having trifluralin, chlorpyrifos, and atrazine as active ingredients. Several rosins, waxes, and synthetic polymers were tested as matrix materials for these three pesticides, and examples of other actives mixed with lignins, cyclodextrins, flours, and starches were mentioned.

Preparation of a controlled-release (CR) formulation of imidacloprid using alkali lignins as a matrix has been reported, and it was found that the release rate of imidacloprid was highly dependent on particle size (*8*). Preparation and CR properties of polymeric microparticles containing alachlor and metolachlor have also been reported (*9*).

Tebuconazole and chlorothalonil were successfully incorporated in polymeric nanoparticles with a median particle diameter of 100−250 nm (*10*). Poly(vinylpyridine) and poly(vinylpyridine-*co*-styrene) were employed as the polymer matrix. The release of tebuconazole into water from the nanoparticle showed the controlled-release character.

The objective of this research project was to develop a CR formulation of tebuconazole that could minimize the initial release of the AI and extend release slowly overtime. CR formulations for ST application need to be free of organic solvent, because of the phytotoxicity issues associated with most solvents. The particle size needs to be small enough so that the formulation does not clog the equipment used during the ST process. Particles of <100 μm function well in seed coatings because they adhere better to seed surfaces than larger particles (*11*). Microencapsulation, where the active ingredient is evenly distributed in a matrix microparticle, would provide an organic solvent-free formulation, with small particles. The matrix of these particles is composed of an organic polymer, and the release rate of AIs from these particles could be controlled by the proper choice of the matrix composition. Matrix microparticle based formulations could be applied onto the seed directly without further processing. The polymers used as the matrix, in this study, include poly(methyl methacrylate) (PMMA) and poly(styrene-*co*-maleic anhydride) (PSMA). Structures and acronyms for different materials used in the preparation of the matrix microparticle are provided in **Table 1**.

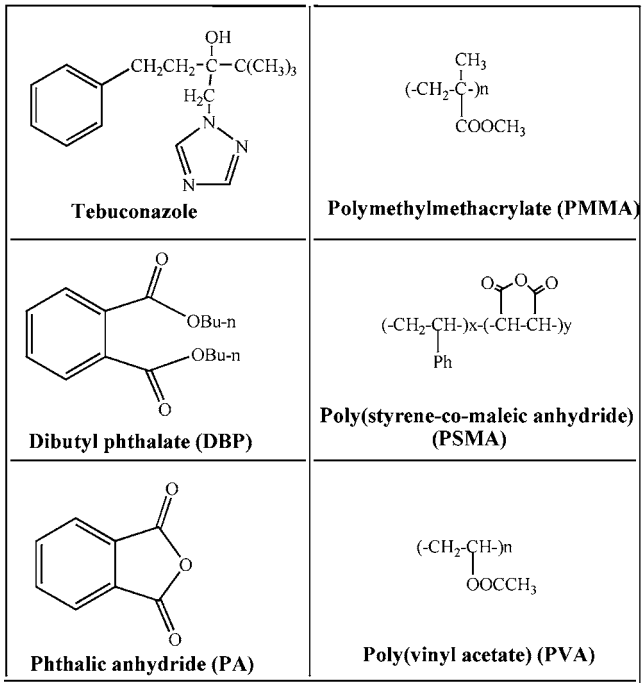
In this paper the preparation and characterization of microparticles containing tebuconazole, its release kinetics in water, and its bioefficacy in controlling wheat rust are investigated in spring wheat. Microencapsulation is achieved by the oil-in-water emulsion process.

MATERIALS AND METHODS

PMMA with molecular weight of 120,000, poly(vinyl acetate) (PVA), PSMA, phthalic anhydride (PA), and dibutyl phthalate (DBP) were used as received from Aldrich. PMMA with molecular weight of 350,000 was used as received from Polyscience. Hydroxypropyl cellulose (Methocel A15LV) was used as received from Dow Chemical Co. Bayer AG provided the technical grade tebuconazole (lot 2786790) with a purity of 98.5%. Soil used for testing was a 1:1 mixture of Dupo silt and Metro Mix 200. Dupo silt contained ~70% silt, ~20% sand, and ~10% clay. Organic matter in this soil was ~5%. Metro Mix 200 was a potting medium produced by Scotts that was composed of peat moss, vermiculite, perlite, horticulture sand, starting fertilizer, and wetting agent. Osmocot, a slow release fertilizer (19−6−12), was a product of Scotts.

Preparation of Polymeric Microparticles. Microparticles were prepared by the oil-in-water emulsion process. The oil phase consisted of tebuconazole, the polymer, and the plasticizers dissolved in methylene chloride, which was easily evaporated to produce suspended droplets in water. PMMA and PSMA were used as the polymer matrix; PVA, PA, and DBP were used as adjuvants to modify the matrix composition and alter the release profile of tebuconazole. Hydroxypropyl cellulose was used as the emulsifier. Emulsion was prepared by mixing the organic and the aqueous phases with a mixer at a high speed. Both the organic and the aqueous solutions were cooled to 4 °C prior to mixing to minimize the loss of methylene chloride. The emulsion formed in this process was stirred at room temperature overnight or until the organic solvent was removed. The product was collected as a white slurry. The weight of the formulated tebuconazole would vary depending on the amount of water evaporated along with methylene chloride in the process. Slurry, microparticles suspended in water, was directly used for the bioefficacy studies.

Table 1. Chemical Structures of Tebuconazole, Matrix Polymers, and Additives



In a typical example, 1.09 g of hydroxypropyl cellulose was dissolved in 89.71 g of water in a 400 mL beaker with mild stirring and heat. The aqueous solution was then cooled in an ice bath to 4 °C. In a separate beaker an organic solution was prepared by dissolving 6.70 g of technical grade tebuconazole and 9.80 g of PMMA (120,000 weight-average molecular weight) in 93.50 g of methylene chloride. The organic solution, cooled to 4 °C, was then poured into the aqueous solution over a period of ~30 s while the mixture was being agitated with a high shear mixer (Silverson model L4R equipped with a six-hole screen), set at high speed. Mixing was continued for 3 min until the mixture formed a milky emulsion. The emulsion was then stirred with a standard mechanical stirrer at room temperature. Methylene chloride was slowly evaporated from the emulsion, to give solid particles dispersed in water, stabilized by hydroxypropyl cellulose. After 20 h, 92.83 g of white slurry was collected. The average particle size for the microparticles was ~8.6 μm.

All of the formulations reported here were prepared following this methodology, but different amounts of adjuvants such as PA, DBP, and PVA were added in the oil phase along with the PMMA. The amount of tebuconazole used in this study was kept constant at 40% based on the total weight of matrix microparticle, and the polymer along with any adjuvant accounted for 60 wt % of the microparticle. PSMA was also used as the polymer matrix. PA, DBP, and PVA were used as additives in the matrix to regulate the release of tebuconazole. The amount of PVA and PA used in the microparticles ranged from 10 to 50% based on the polymer content in the matrix, whereas, the amount of DBP ranged from 1 to 10%. The characteristics of the microparticles made from the solvent evaporation process are summarized in **Table 2**.

Encapsulation Efficiency. The encapsulation efficiency of tebuconazole CR formulations was tested by mixing a known amount of

Table 2. Characteristics of the Microparticles Based on PMMA Matrix Modified with Low Molecular Weight Plasticizers, PA and DBP

polymer/ plasticizer formulation[a]	mean diameter (μm)	REA (%)	encapsulation efficiency(%)
PMMA 350	13.5	2.7	97.3
PMM 350/PA-10	13.4	2.8	97.2
PMMA 350/PA-30	6.4	3.3	96.7
PMMA 350/PA-50	4.7	3.5	96.5
PMMA 120	8.6	2.8	97.2
PMMA 120/PA-50	4.0	4.8	95.2
PMMA 120/DBP-1	8.1	1.9	98.1
PMMA 120/DBP-5	8.5	1.7	98.3
PMMA 120/DBP-7	8.2	2.2	97.8
PMMA 120/DBP-10	7.5	3.1	96.9

[a] Numbers after the PMMA denote the molecular weight of the PMMA, and numbers after the plasticizers, PA or DBP, denote the amount (percent) of plasticizer used in the microparticle. The amount of tebuconazole was kept constant at 40% based on the total weight of the microparticle. Readily extractable actives (REA) is the amount of unincapsulated tebuconazole as measured by HPLC. Particle sizes reported here were measured by a Coulter LS130.

the formulation with water at room temperature. The total concentration of tebuconazole in the aqueous mixture was no more than one-third of its water solubility (32 mg/L). The mixture was then shaken for 1 min and immediately filtered through a 0.45 μm PTFE filter. The amount of AI in the filtered solution was determined by reverse phase HPLC, using an Alltech Alltima C18 column (5 μm particle size; 250 $\times$ 4.6 mm) and UV detection at 220 nm. The mobile phase consisted of 3.8 g of potassium phosphate monobasic plus 1.5 L of water adjusted to pH 2.5 with 85% phosphoric acid plus 2.5 L of acetonitrile. The injection volume was 100 μL, and the flow rate was 1.2 mL/min. No interference with any other formulation components was observed using this analysis system. The filtered amount represents readily extractable active ingredient (REA), and encapsulation efficiency is expressed as 100 $-$ %REA.

Release Study of Tebuconazole. The release rate of tebuconazole from microparticles was tested by mixing a known amount of the formulation with water as described under Encapsulation Efficiency. The mixture was then placed in a closed container and agitated at room temperature by a magnetic stirrer (no heat). At various time intervals aliquots of the mixture were removed and filtered through a 0.45 μm PTFE filter. The amount of AI in the filtered solution was determined by reverse phase HPLC, as described under Encapsulation Efficiency.

Measurement of Particle Sizes and Size Distributions. *Particle Size Determination Using Scanning Electron Microscopy (SEM).* The microparticles were diluted with distilled water and dispersed onto polished aluminum SEM stubs. The stubs were air-dried prior to sputter coating with Au/Pd in a Polaron E5100 for 45 s, pulsed at 1 s intervals, to provide electron beam conductivity. The scanning electron micrographs were obtained using a JEOL 840 scanning electron microscope at an accelerating voltage of 5 kV and a probe current of 100 pA.

Particle Size Determination Using the Coulter Counter. The microparticles were diluted with distilled water and then added to the sample vessel of a Coulter LS130 with a fluid module. Particle sizes and their distribution were measured by a light scattering method using a laser at 750 nm.

Measurement of Thermal Properties. Glass transition temperatures (T_g), heat of fusion, and melting temperatures were measured by differential scanning calorimetry (DSC) on a Perkin-Elmer DSC-7. Approximately 10 mg samples were encapsulated in standard aluminum DSC pans and heated from $-$20 to 140 °C. Heating and cooling rates were 20 °C/min. The midpoint of the total change in heat capacity was designated the glass transition, and the peak temperature was designated the melting point. All calculations were performed on the first heating cycle. A pure indium metal was used to determine the temperature correction factor.

Bioefficacy of Controlled-Release Formulations. Spring wheat seeds (Fortuna variety) were planted in sterile Dupo silt loam supplemented with Osmocote (slow release fertilizer) and covered with a 1:1 blend of sterile Dupo silt loam and Metro-Mix 200 potting medium. CR formulations were injected into the soil near the seeds 11 days after planting (DAP), using a 1 mL syringe with a blunt end pippetting needle. A commercial formulation of tebuconazole, Raxil, was also injected for comparison. Some plants were left untreated to serve as the untreated control (UTC), and another group of plants were treated with a standard commercial spray of tebuconazole (foliar application with Raxil formulation). All of the CR formulations were applied at rates of 100 and 200 g of tebuconazole/100 kg of wheat seed. Raxil, whether applied in-furrow or foliar, was applied at the standard rate of 100 g of AI/100 kg equivalent of seed.

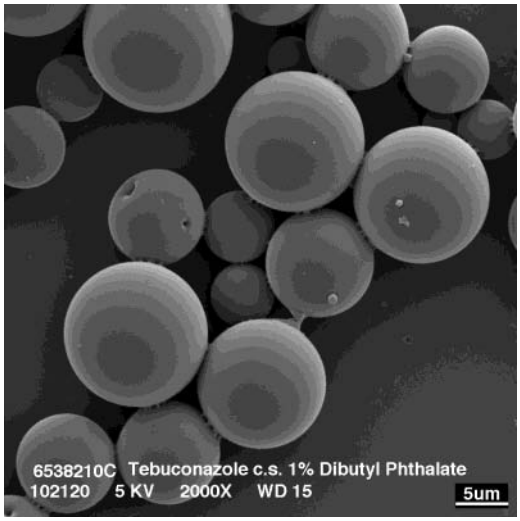


Figure 1. SEM image of PMMA/DBP microcapsules containing 1% DBP.

Wheat plants were inoculated with wheat root fungus 29 DAP, 18 days after injection of CR formulations. The spores were collected from an infected in-house culture of Caldwell winter wheat. On the 10th day after inoculation, wheat rust infection was rated on the third leaf of each plant, by visually estimating the area of leaf covered with sporulating lesions.

RESULTS AND DISCUSSION

Preparation and Characterization of the Microparticles Containing Tebuconazole. Table 2 shows the characteristics of microparticles based on PMMA. The matrix was modified with different amounts of low molecular weight plasticizers, PA and DBP. All of the formulations were analyzed for tebuconazole content. The encapsulation efficiency of tebuconazole in the microparticles was determined by measuring the unencapsulated amount of tebuconazole by HPLC. Encapsulation efficiency ranged from 95.2 to 98.3%, which demonstrates that the solvent evaporation method was very efficient, with the majority of tebuconazole successfully encapsulated in the microparticles.

Particle size and size distributions of the suspension were measured by a Coulter LS130. The size and shape of the microparticles were also determined by SEM. The average particle sizes measured by the Coulter LS130 ranged from 3.5 to 13.5 μm. Size distributions were found to be unimodal in all cases. **Figure 1** shows a SEM micrograph of a formulation with microparticles derived from PMMA 120 matrix containing 1% DBP. This SEM is typical of all the formulations prepared in this study. Microparticles had a spherical shape with a smooth surface. However, small pits on the surface of the spheres were also observed. The size of the microparticles, as observed by SEM, ranged from 2 to 16 μm with the majority being in the size range from 5 to 10 μm. This agrees well with the size range measured by the Coulter LS130 (**Table 2**).

Table 3. Characteristics of Microparticles Based on Polymer Blends: PMMA and PSMA Matrices Modified with PVA

polymer blend formulation[a]	mean diameter (μm)	REA (%)	encapsulation efficiency(%)
PMMA 120	8.6	2.8	97.2
PMMA 120/PVA-10	5.7	2.5	97.5
PMMA 120/PVA-20	5.5	3.4	96.6
PMMA 120/PVA-30	5.2	3.0	97.0
PSMA	3.5	5.2	94.8
PSMA/PVA-30	7.4	4.8	95.2

[a] Numbers after the PMMA denote the molecular weight of the PMMA, and numbers after the PVA denote the amount (percent) of PVA used in the microparticle. The amount of tebuconazole was kept constant at 40% based on the total weight of the microparticle. Readily extractable actives (REA) is the amount of unincapsulated tebuconazole as measured by HPLC. Particle sizes reported here were measured by a Coulter LS130.

SEM micrographs also showed the presence of crystals in some preparations. It is suspected that these crystals are derived from unencapsulated tebuconazole. HPLC analysis suggested that a small amount of tebuconazole, ranging from 1.7 to 4.8%, was not encapsulated. As solubility of tebuconazole is only 32 mg/L at room temperature, it is unlikely that the crystals are derived from the dissolved tebuconazole. Because the total surface area of the matrix is very large (due to the small size of the microparticle) the majority of the unencapsulated tebuconazole may have come from the surface of the microparticles.

Differences in the particle size of different formulations (**Table 2**) are mainly due to the differences in the viscosity of the organic phase. The average particle size for the formulation derived from PMMA 350 is 13.5 μm. In contrast, PMMA 120 produced microparticles in the range of 8.6 μm under the same experimental conditions, probably due to lower viscosity. Addition of the low molecular weight compound, PA, in the organic phase, lowered the viscosity of the organic phase, resulting in emulsions with small particle size. Lowering of the viscosity and the reduction in the particle size by the addition of PA was observed with both PMMA 350 and PMMA 120 (**Table 2**). In the PMMA 120/DBP system, the addition of DBP did not affect the particle size at least up to 7% DBP. When the amount of DBP was increased to 10%, the particle size changed from 8.6 to 7.5 μm. Matrices based on PSMA and PMMA 120 were modified with PVA, and the characteristics of the microparticles are reported in **Table 3**. A significant change in particle size of PMMA 120 was not observed because the matrix was modified using a polymeric additive, PVA (**Table 3**).

Release Kinetics of Tebuconazole in Water. Figure 2 shows the release rate of tebuconazole from the PMMA-based matrix modified with different amounts of PA. The amount of PA in the microparticles played an important role in controlling the release of tebuconazole. Formulations with PA exhibited a faster release rate of tebuconazole than the formulation without PA; the release rate increased as the amount of PA in the matrix was increased. Microparticles containing 50% PA released 70% tebuconazole after 500 h, whereas microparticles without PA released only 17% tebuconazole in the same period.

Increase in the release rate of tebuconazole, on addition of PA, may in part be due to the decrease in the particle size, which accompanies the addition of 30% and higher amounts of PA to the PMMA matrix. It is interesting to note, however, that even though addition of 10% PA did not affect the particle size, it did increase the release rate.

PA was used as an additive to modify the PMMA matrix because it was hypothesized that in the presence of water PA would convert to the more hydrophilic acid form and create "hydrophilic channels" in the matrix. Consequently, the release rate of tebuconazole would increase if these hydrophilic channels were created. Although these assumptions may be valid, no evidence of channels was observed in SEM micrographs.

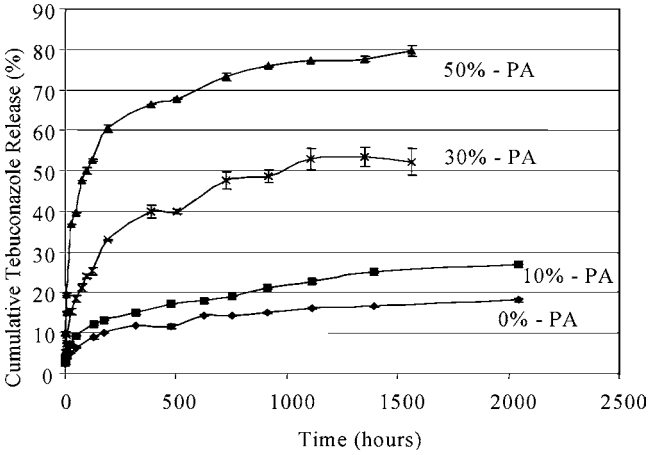


Figure 2. Cumulative release of tebuconazole from PMMA 350/PA microparticles containing 0, 10, 30, and 50% PA. Release of tebuconazole from matrix microparticle into water was measured under infinite sink conditions.

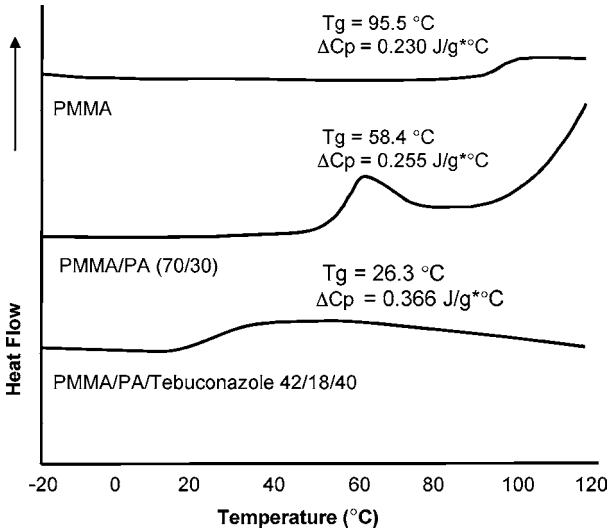


Figure 3. DSC thermograms of (1) PMMA, (2) PMMA/PA, and (3) PMMA/PA/tebuconazole. Glass transition temperatures (T_g), heats of fusion, and melting temperatures were measured on a Perkin-Elmer DSC-7, at heating and cooling rates of 20 °C/min.

Microparticles were analyzed by thermal analysis to determine whether PA acts as a plasticizer, which would reduce the glass transition temperature (T_g) of the resulting matrix. A matrix with a lower T_g could be expected to release tebuconazole more rapidly due to increased free volume (*12*). DSC thermograms of PMMA, PMMA/PA, and PMMA/PA/tebuconazole are shown in **Figure 3**. The T_g of PMMA was reduced to 58.1 from 96 °C when PMMA was mixed with PA in a 70/30 weight ratio. When a mixture of PMMA and PA (70/30) was blended with tebuconazole in a 60/40 weight ratio, the T_g was further reduced to 26.6 °C (**Table 4**); this is the T_g of the matrix microparticle PMMA 350/PA-30 containing 40% tebuconazole. These results clearly indicate that PA and its mixtures with tebuconazole act as a plasticizer for PMMA. Addition of PA, therefore, leads to a higher free volume of the matrix and increased speed of tebuconazole release.

Table 4. Glass Transition Temperature (T_g) and/or Melting Point for Plasticizers, Matrix Polymers, and Controlled-Release Formulations[a]

sample	formulation	T_g (°C)	melting temp (°C)
PMMA 120		96.0	
phthalic anhydride (PA)			136.2
tebuconazole			105.0
PMMA/PA-30		58.1	
PMMA/PA/tebuconazole (42/18/40 wt ratio)	PMMA 350/ PA-30	26.6	93.4
PVA		27.1	
PMMA/PVA/tebuconazole (48/12/40 wt ratio)	PMMA 120/PVA-20	25.5	

[a] T_g, heat of fusion, and melting temperatures were measured on a Perkin-Elmer DSC-7, at heating and cooling rates of 20 °C/min.

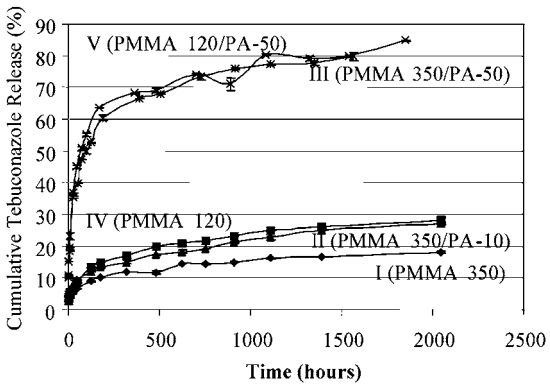


Figure 4. Effect of the molecular weight of polymer matrix on the release profile. Cumulative release of tebuconazole from PMMA 350/PA microparticles containing (I) 0%, (II) 10%, and (III) 50% PA compared with PMMA 120/PA with (IV) 10% and (V) 50% PA. Release of tebuconazole from matrix microparticle into water was measured under infinite sink conditions.

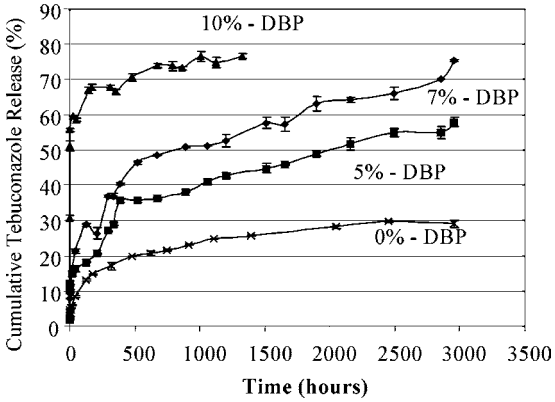


Figure 5. Cumulative release of tebuconazole from PMMA 120/DBP microparticles containing 0, 5, 7, and 10% DBP. Release of tebuconazole from matrix microparticle into water was measured under infinite sink conditions.

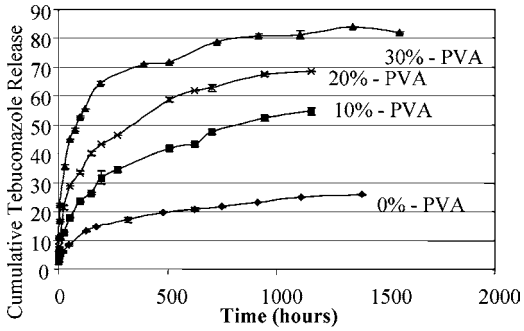


Figure 6. Cumulative release of tebuconazole from microparticles with PMMA 120/PVA containing 0, 10, 20, and 30% PVA. Release of tebuconazole from matrix microparticle into water was measured under infinite sink conditions.

Figure 4 shows the effect of the molecular weight of the matrix on the release profile. Release from the matrix with a 350,000 MW is much slower compared to a matrix made from PMMA with 120,000 MW. Addition of ~10% PA into the PMMA 350 matrix increased the release rate to a level approximating the release rate from the PMMA 120 matrix. At a higher concentration, 50% of PA, tebuconazole release rate was influenced more by the plasticizer than the polymer molecular weight; the release profile of tebuconazole from PMMA 120/PA-50 (**Figure 4**, curve V) was similar with PMMA 350/PA-50 microparticles (**Figure 4**, curve III).

Because modification of the release profile in the PMMA/PA-based microparticle is attributed to the fact that PA functions as a plasticizer, a logical step was to test traditional plasticizers. Phthalate esters are a well-known group of plasticizers (*13*), and so we decided to modify the PMMA matrix with DBP. Microparticles containing different amounts of DBP were prepared in a similar fashion as described for the PMMA/PA-based microparticles.

Figure 5 shows the release rate of tebuconazole into water from matrix microparticles with PMMA 120 as the matrix polymer and DBP as the plasticizer at levels ranging from 0 to 10%. Addition of DBP in PMMA 120 matrices increased the release rate of tebuconazole. The release rate increased as the amount of DBP in the matrix microparticle was increased. The trend is consistent with the earlier observations when PA was used as an additive in a PMMA matrix. However, a "burst release" (*14*) was observed when DBP content was increased to 10%; 50% of tebuconazole was released in 3 h. These results suggest that the release profile of PMMA 120-based matrix microparticles is more influenced by DBP than PA and that the addition of 10% DBP radically changes the release profile.

Polymeric matrices can also be modified by the use of polymer blends (*12*). The PMMA 120 matrix was modified with different amounts of PVA. Release profiles from the microparticles based on PMMA 120 are compared with the PMMA 120/PVA-based microparticles in **Figure 6**. Addition of the PVA in a PMMA matrix increased the release rate of tebuconazole, and the rate is further increased by increasing the concentration of PVA in the matrix.

Although the addition of 10% PVA in a PMMA matrix decreased the particle size from 8.6 to 5.7 μm, further addition PVA did not appreciably affect the particle size. The release rate, however, kept on increasing as the amount of PVA in the matrix was increased. It therefore appears that the increase in the rate of tebuconazole release is more related to the nature of the matrix than merely the particle size. To study the thermal behavior of PMMA matrix modified with PVA, a blend of PMMA/PVA/tebuconazole was analyzed by DSC, and results are shown in **Table 4**. The T_g of the PMMA matrix was found to decrease when it was blended with PVA, a polymer with a low T_g (27 °C) and no crystallinity.

Figure 7 shows the release from matrix microparticles prepared with PSMA and a 70/30 PSMA/PVA blend. The release rate of tebuconazole from the PSMA matrix was also increased by the addition of PVA.

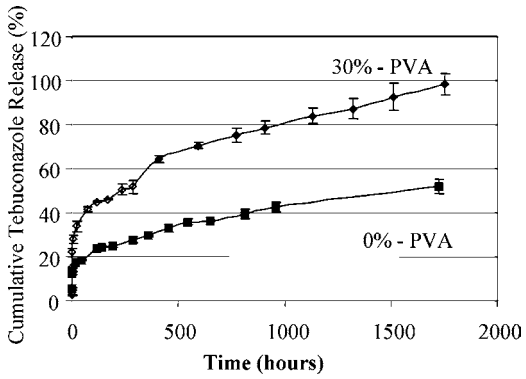


Figure 7. Cumulative release of tebuconazole from PSMA/PVA microparticles containing 0 and 30% PVA. Release of tebuconazole from matrix microparticle into water was measured under infinite sink conditions.

Table 5. Constants from Fitting the Generalized Model, $M_t/M_z = kt^n + C$, to the Release Data of Tebuconazole from Microparticles Modified with PA and DBP[a]

formulation	$k \times 10^2$	n	$C \times 10^2$	r	t_{50} (h)
PMMA 350	1.30	0.34	–0.501	0.9897	47700
PMMA 350/PA-10	1.62	0.36	–0.39	0.9956	14000
PMMA 350/PA-30	4.23	0.36	1.26	0.9943	889
PMMA 350/PA-50	8.70	0.37	3.27	0.9900	94
PMMA 120/PA-50	8.90	0.39	3.82	0.9941	68
PMMA 120	1.98	0.35	–0.999	0.9925	10740
PMMA 120/DBP-5	2.942	0.36	3.766	0.9821	2104
PMMA 120/DBP-7	5.923	0.31	–0.165	0.9858	984
PMMA 120/DBP-10[b]					3

[a] r is a correlation coefficient, k is the constant that incorporates the matrix properties, n is a diffusional parameter, C is a constant, and t_{50} is the time it takes to release 50% of tebuconazole. [b] The release of the active was very fast, and the release curve does not fit the model.

Table 6. Constants from Fitting the Generalized Model, $M_t/M_0 = kt^n + C$, to the Release Data of Tebuconazole from Microparticles Modified with PVA[a]

formulation	$k \times 10^2$	n	$C \times 102$	r	t_{50} (h)
PMMA 120	1.98	0.35	–0.999	0.9925	10740
PMMA 120/PVA-10	4.13	0.37	–1.51	0.9958	915
PMMA 120/PVA-20	6.68	0.35	–1.08	0.9963	334
PMMA 120/PVA-30	11.01	0.34	–1.04	0.9959	91
PSMA	2.14	0.40	8.63	0.9877	2229
PSMA/PVA-30	17.18	0.19	2.00	0.9879	228

[a] r is a correlation coefficient, k is the constant that incorporates the matrix properties, n is a diffusional parameter, C is a constant, and t_{50} is the time it takes to release 50% of tebuconazole.

Release data were analyzed by applying the empirical equation

$$M_t/M_z = kt^n + C$$

where M_t/M_z is the amount of tebuconazole released at time t, k is the constant that incorprates the matrix properties, the exponent n is a diffusional parameter, which is indicative of the transport mechanism, and C is a constant.

The values of k, C, and n obtained from initial 60% release of tebuconazole are summarized in **Tables 5** and **6**. The correlation coefficient (r) is >0.98 for all of the formulations described and indicates that there was an excellent correlation of tebuconazole release profile from microparticles, using the empirical equation above. The diffusion parameter equal to 0.50 corresponds to Fickian diffusion ($y = kt^{1/2}$) from one-dimensional matrices (*6*). For spheres, when corrected for the geometry of the device (microparticle), the diffusion parameter changes to a value of 0.43 when Fickian diffusion occurs in a spherical monolithic matrix. Values of n close to 0.43 are indicative of Fickian diffusion (*6*).

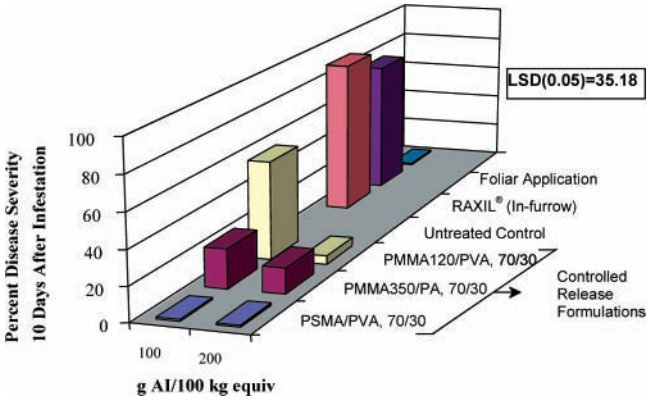


Figure 8. Disease severity of wheat rust on third leaf 28 days after CR soil injection.

As evidenced in **Table 5**, the release from PMMA, irrespective of MW, was controlled by Fickian diffusion. Addition of PA did not significantly change the value of n. The time it takes to release 50% of tebuconazole from the matrix, defined as t_{50}, however, changed considerably. Addition of 50% PA into a PMMA 350 matrix reduced the t_{50} value from 47.7×10^3 h to only 94 h. Also, the effect of DBP on the release profile of the matrix microparticle was much more pronounced than that of PA. The t_{50} value was changed from 10.74×10^3 to 9.84×10^2 h when the PMMA 120 matrix was modified with 7% DBP. When the amount of DBP was increased to 10%, the mechanism of release was changed from diffusion control to a "burst release", where the t_{50} was reduced from 10.74×10^3 h to only 3 h. Matrices made from miscible polymer blends also followed Fickian diffusion, where t_{50} was reduced as the amount of low T_g polymer component (PVA) was increased in the matrix (**Table 6**).

Bioefficacy and Length of Control. Bioefficacy of the polymer-based CR formulations was evaluated using a bioassay that assessed the severity of wheat rust on the third true leaf of wheat, 10 days after infestation. CR formulations PMMA 350/PA-30, PSMA/PVA-30, and PMMA 120/PVA-30 (described in **Figures 2**, **6**, and **7**) were used for bioefficacy studies. These three formulations were chosen because they were found to have intermediate rate of release in water with t_{50} values ranging from 91 to 889 h; unmodified matrices based on PMMA or PSMA release too slowly and have been found to be ineffective in controlling diseases in previous experiments. **Figure 8** shows disease severity of wheat rust 10 days after infestation. The three CR formulations provided significantly better protection against wheat rust than the Raxil in-furrow treatment. The PSMA/PVA-30 formulation provided the highest level of protection against rust among the CR formulations. Efficacy of the controlled-release formulations was greater at the 200 g dose than at the 100 g dose. The degree of protection provided by PSMA/PVA-30 at the 200 g/100 kg rate was equal to the protection provided by the foliar application of tebuconazole. This indicates good potential for an effective CR formulation applied as an in-furrow application.

Conclusion. Polymer-based controlled-release formulations of tebuconazole were prepared using the oil-in-water emulsion process with high encapsulation efficiency. The release of

tebuconazole from the microparticles was found to be a diffusion-controlled process. The release rate of tebuconazole increased when plasticizers and PVA were added to the matrix microparticle. Increased release rate was achieved because the addition of plasticizers and PVA lowered the T_g of the matrix. DBP was found to influence tebuconazole release from the PMMA matrix more dramatically than other additives. Addition of 10% DBP in PMMA changed the release from a diffusion-controlled process to burst release. Effective CR formulations injected into the soil provided the same level of protection against wheat rust as a foliar application of tebuconazole.

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LITERATURE CITED

(1) Brandl, F. Seed treatment technologies: Evolving to Achieve Crop Genetic Potential. *BCPC Symp. Proc.* **2001**, *76*, 3−18.

(2) Tomlin, C. *The Pesticide Manual*; British Crop Protection Council: Surrey, U.K., 1997.

(3) Markus, A. Advances in the Technology of Controlled-Release Pesticide Formulations. In *Microencapsulation: Methods and Industrial Applications*; Benita, S., Ed.; Dekker: New York, 1996; pp 73−91.

(4) Washington, C. Drug Release from Microparticulate Systems. In *Microencapsulation: Methods and Industrial Applications*; Benita, S., Ed.; Dekker: New York, 1996; pp 155−182.

(5) Bahadir, M. Controlled Release of Pesticides. In *Controlled Release, Biochemical Effects of Pesticides, Inhibition of Plant Pathogenic Fungi: Chemistry of Plant Protection 6*; Bowers, W. S., Eds.; Springer-Verlag: Berlin, Germany, 1990; pp 1−64.

(6) Ritger, P. L.; Peppas, N. A. A Simple Equation for Description of Solute Release I. Fickian and Anomalous Release from Non-Swellable Devices in the Form of Slabs, Spheres, Cylinders or Discs. *J. Controlled Release* **1987**, *5*, 23−36.

(7) Park, D. J.; Jackson, W. R.; McKinnon, I. R.; Marshall, M. Controlled Release of Pesticides from Microparticles. In *Controlled-Release Delivery Systems for Pesticides*; Scher, H. B., Ed.; Dekker: New York, 1999; pp 89−136.

(8) Fernandez-Perez, M.; Gonzalez-Predas, E.; Urena-Amate, M. D.; Wilkins, R. M.; Lindrup, I. Controlled Release of Imidacloprid from a Lignin Matrix: Water Release Kinetics and Soil Mobility Study. *J. Agric. Food Chem*. **1998**, *46*, 3828−3834.

(9) Dowler, C. C.; Dailey, O. D., Jr.; Mullinix, B. Polymeric Microparticles of Alachlor and Metolachlor: Preparation and Evaluation of Controlled-Release Properties. *J. Agric. Food Chem*. **1999**, *47*, 2908−2913.

(10) Liu, Y.; Yan, L.; Heiden, P.; Laks, P. Use of Nanoparticles for Controlled Release of Biocides in Solid Wood. *J. Appl. Polym. Sci.* **2000**, *79* (3), 458−465.

(11) Asrar, J.; Ding, Y. Unpublished results, 2001.

(12) Asrar, J.; Essinger, J. F., Jr. Controlled Release Formulations and Methods for Their Production and Use. WO 0221913 A2, 2002.

(13) Billmeyer, F. W., Jr. In *Textbook of Polymer Science*, 2nd ed.; Wiley: New York, 1971; 501 pp.

(14) Huang, X.; Brazel, C. S. On the Importance and Mechanisms of Burst Release of in Matrix-Controlled Drug Delivery Systems. *J. Controlled Release* **2001**, *73* (2), 121−136.

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