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A SIMPLIFIED ANALYTICAL PROCEDURE FOR THIABENDAZOLE IN CITRUS DRENCHER SUSPENSIONS

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Abstract. A simplified procedure is described for the estimation of thiabendazole (2- (4-thiazolyl)-benzimidazole) in pallet box drenchers. The method is useful in the concentration range of 200 to 1800 ppm without dilution. Suspensions with a higher concentration may be diluted with water. In this method, a representative sample of treating material and sodium hydroxide is added to a screw top culture tube containing a chlorinated hydrocarbon solution of cupric acetate and 1-dimethyl-amino-2-propanol. The mixture is shaken and then allowed to stand while the phases separate. The chlorinated hydrocarbon layer is read on a portable colorimeter. Total time from sampling to result can be less than 10 minutes. Several chlorinated hydrocarbons can be used.

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Treating fruit with a fungicide before degreening is an effective method for controlling both *Diplodia* stem-end rot and green mold. Since delays of more than 24 hr between harvest and treatment can seriously reduce the effectiveness of the treatment (3), pallet box drenchers are becoming common in Florida (7).

Two types of drencher are currently in use, traditional drenchers and truck drenchers. In the traditional drencher, pallet boxes are loaded on a conveyor which carries them under a manifold that floods fungicide suspension onto the boxes (6). In a truck drencher the load is left on the truck which is driven into the applicator which floods the suspension over the load (7, 8). In either case the suspension is collected in a sump for recirculation.

Until the 1989-90 citrus packing season, the fungicide of choice for use in drenchers was benomyl as it was relatively inexpensive and easy to use. With the withdrawal of postharvest uses of benomyl, thiabendazole (TBZ) has become the only practical fungicide available for this application (5, 16). While imazalil will work in drenchers, its use in Florida is limited by its higher cost and lower effectiveness against *Diplodia* stem-end rot compared to TBZ (3, 4, 5).

Both TBZ and benomyl are insoluble materials, and in an aqueous system there is a tendency to settle out. There is also a tendency for the fungicide to remain on the fruit

and equipment passing through, leaving behind the water (15). This, along with the fact that for the most part drenchers are outdoors and subject to rain, makes maintaining the suspension concentration difficult (14, 17). In the case of benomyl, the operator normally would use more material than was necessary for good decay control, and drain and recharge the drencher often. With TBZ two factors have made this less practical. The first is the greater cost of TBZ and the second is that changing regulations have made the disposal of large volumes of spent fungicide suspension more difficult.

These factors have made it desirable to have a quick method of determining the concentration of TBZ in the drencher suspension. In the past, the normal method was to send the sample to a laboratory where the sample could be analyzed using an ultraviolet (UV) spectrophotometer (10). In 0.1 N HCl TBZ exhibits an absorbance peak at 302.5 nm which varies with the concentration according to Beer's law. A major problem with this procedure was that by the time the concentration was determined, several days may have passed and many hundred boxes of fruit processed.

The need for more timely results has resulted in the development of several field test methods (1, 10, 11, 12). In Australia, it is reported that the analyst and his equipment go to the packinghouse to perform the tests (9).

Each of these has various drawbacks. A UV spectrophotometer is a delicate and expensive instrument. Sending it along with a highly trained analyst to a packinghouse is expensive and impractical. The other methods proposed use chemical apparatus (pipettes and separatory funnels) not normally found in a packinghouse. They also involve the handling of hazardous chemicals and the subjective comparison of color standards with a degree of skill that would require a trained technician. Some methods (11, 12) called for the use of radioactive reagents. All of the current methods would require a person trained beyond the normal requirements of a citrus packer, and unless there were other duties that person could perform, their expense would be prohibitive.

In order to be useful to the Florida citrus packer, a method would have to be simple enough that an average person with minimal training could get meaningful results. In addition to this, worker safety and the time taken to get results are considerations. A modification of the methods by Miller, et al. (11, 12) was found to meet these requirements to a satisfactory degree.

The solvent chosen by Miller et al. was chloroform. This solvent has some objectionable properties that make a substitute desirable. Several commonly available solvents were investigated to determine their usefulness in this procedure, and their properties as reported in standard reference works, are summarized in Table 1 (13, 18). An acceptable material would have to be a good solvent for the thiabendazole-copper-amine (TCA) complex, should be non-flammable, give good separation of phases, have a low level of toxicity as measured by the amount of vapor allowed in the air of the work space (TLV), and a low vapor pressure. Of those reviewed, carbon tetrachloride was rejected because of its very low TLV. Tetrachloroethylene, 1,1,1 trichloroethane and trichloroethylene proved to be weak solvents for TCA and were excluded from further tests.

Table 1. Properties of selected solvents.

Solvent	Sp.Gr. ^z	B.P. ^y	TLV. ^x	V.P. ^w
Carbon Tetrachloride	1.60	77	10	100/23
Chloroform	1.48	61	25	100/10
Methylene Chloride	1.34	40	200	380/22
Tetrachloroethylene	1.62	121	100	16/22
1,1,1 Trichloroethane	1.33	74	100	100/20
1,1,2 Trichloroethane	1.44	114	- ^v	40/35
Trichloroethylene	1.47	87	100	100/32

^zSpecific gravity of liquid as compared to water.

^yBoiling point in °C at normal pressure.

^xThreshold Limit Value.

^wVapor Pressure, mm at temperature in °C.

^vTLV not available. Not in standard reference works.

Materials and Methods

A chlorinated hydrocarbon solvent is prepared by dissolving 1.17 g copper acetate monohydrate and 20 ml reagent grade 1-dimethylamino-2-propanol per liter. A 10 ml aliquot of copper-amine reagent is added to a 16 by 150 mm screw top glass culture tube (Kimble, Kimax 45066 or equivalent). Five ml of a well mixed drencher sample and 1 ml 1 N sodium hydroxide solution are added to the tube. The tube is closed and then mixed by shaking for 3 minutes. The tube is placed in an upright position for 5 minutes, or until the phases separate. The absorbance or transmittance of the solvent layer is determined at 420 nm using a portable colorimeter with the pure solvent as a reference.

For these trials a Hach DR-100 colorimeter with a fixed 420 nm filter was used. It was modified to take a 16 mm tube. The concentration is then determined by using a standard curve. Complete separation may not occur after 5 min, but under average conditions, the clarity of the solvent layer will be such that consistent results may be obtained.

Samples of a TBZ dispersion were prepared by diluting a concentrated dispersion and then analyzed by a UV spectrophotometer by comparing absorbance at 302.5 nm with a standard curve. These samples were subsequently subjected to the simplified procedure using Chloroform and 1,1,2 Trichloroethane to produce Table 2. All transmittances were read after 5 min.

Before reacting with TBZ, the chlorinated hydrocarbon solvent solution has a green to blue green color with

Table 2. TBZ Concentration vs. Transmittance at 420 nm in 1,1,2 Trichloroethane and Chloroform after 5 minutes.

Nominal Concentration	Solvent		
	UV ^z	1,1,2-T ^y	Chloroform ^x
1800 ppm	1782	6.5	4.5
1320 ppm	1357	8	6.5
900 ppm	810	11	14
720 ppm	638	15	20
450 ppm	462	21	28
180 ppm	154	35	45

^zParts per million determined by ultraviolet spectrophotometry at 302.5 nm.

^yTransmittance of 1,1,2 Trichloroethylene layer at 420 nm. after 5 min separation.

^xTransmittance of chloroform layer at 420 nm. after 5 min separation.

an absorbance peak between 355, 387 and 385 nm respectively for Chloroform, 1,1,2 Trichloroethane and methylene chloride, respectively. Upon separation, the tube has a lower slightly cloudy, blue/green colored layer. The upper layer contains the unreacted copper-amine complex and has a deep blue color typical of the aqueous solutions of such compounds. After the reaction, the absorbance peaks for the solvent layer are 405, 402 and 390 nm (Table 3).

After 5 minutes the solvent layer continues to clear, but at inconsistent rates. Filtering the solvent through phase separating filter paper will improve the clarity of the solution (Table 3). While this will improve the precision of the procedure, the extra time, equipment and handling required do not warrant this step.

During 1989-1990 this procedure has been used to control the TBZ concentration at two commercial citrus packinghouses. Several samples have been analyzed at the packinghouse and then at the laboratory. These results are summarized in Table 4.

Results and Discussion

When inexperienced workers attempt to use standard pipettes, inaccuracies of substantial proportions may occur. With the pipetting of suspensions these errors have been found to be as much as 300% (2). In order to minimize the problems inexperienced personnel may have with pipettes, a syringe was used to measure and dispense the TBZ suspensions in these trials. All of the analysis of this procedure, reported in Tables 2, 3 and 4, were by the use of syringe. All of the samples reported in Table 2 were duplicated by pipette. The differences between samples at any given concentration was less than 10%. The syringe used in all of these trials was a disposable 5 ml size (Air-Tite Corp. VA Beach, VA—No. 89 331).

The limits of this procedure for undiluted drench suspensions are between 200 and 1800 ppm TBZ. Below this concentration the cloudiness of the unfiltered layer prevents an accurate transmittance reading. Above this concentration the copper-amine complex becomes exhausted and the light transmittance of the solvent layer becomes so low as to make accurate readings impractical. While this range is adequate for on site quality control of drenchers in Florida, the upper range could be extended by simple dilution. Reaching the upper limits of the procedure can be quickly determined by observing the upper layer. Absence of the typical deep blue copper-amine color indicated that this reagent has been exhausted.

Table 3. Comparison of solvents vs TBZ concentration.

Solvent	Peak ^y	TBZ concentration ^z		
		2106 ppm	1012 ppm	530 ppm
Chloroform	355/405	2.5/6*	11/16	18/33
Methylene Chloride	385/390	7.5/15	12/22	26/40
1,1,2 Trichloroethane	387/402	6.5/16	11/24	24/44

^zAs determined by ultraviolet spectrophotometry at 302.5 nm.

^yWavelength of point of maximum absorbance of TCA in solvent in milimicrons. First figure before reaction, second after reaction.

*Transmittance of chloroform layer at 420 nm. First figure after 5 min separation, second figure after filtering.

Table 4. TBZ concentration in selected samples—Field test vs. Laboratory.

Sample No.	TBZ concentration in ppm.	
	Field ^z	Laboratory ^y
1	1280	1357
2	450	455
3	100	10 ^x
4	950	810
5	1800+	2750

^zChloroform solvent.

^yAs determined by ultraviolet spectrophotometry at 302.5 nm.

^xDrencher operated several days without automatic TBZ equipment running and rain for several days.

The choice of solvent is a matter of availability and operating conditions. Chloroform is the most hazardous and volatile, so that if the technician were to be working in a closed area these would be contraindications to its use. 1,1,2 Trichloroethane has a very low volatility and a high safety factor which would favor its use in an enclosed environment. It has the drawback of separating less cleanly than the others, while methylene chloride separates more cleanly. The major drawback of methylene chloride is that the peak absorbance of the TCA solution is outside the range of most simple filter colorimeters. However, the peak is broad enough that useful results can be obtained.

It should be noted that the meter readings are best taken at a location sheltered from direct sunlight or at least the sample tube should be shaded. Since the sample tube sticks up above the level of the meter too high to allow the closing of the cover, strong sunlight will reflect down into the instrument and give a false reading. In practice, simply shading with a towel or the hand has proved adequate with this equipment.

During the citrus processing season a packer desiring to drench fruit at 1000 ppm TBZ would normally be satisfied if he could maintain the concentration between 750 and 1250 ppm. This procedure meets these requirements of accuracy, it is relatively inexpensive, portable and simple enough to be operated by most packinghouse or service company personnel. In connection with laboratory back up, this simplified procedure can be most useful.

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