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BENLATE RESIDUES IN 'FLORIDA SWEET' CORN AS A RESULT OF SEED TREATMENT[1]

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Abstract. **'Florida Sweet' variety corn seed was treated with Benlate® [benomyl, methyl 1-(butylcarbamoyl)-2-benzemidizolecarbamate] for protection against fungus attack. Husks, cobs and kernels from plants grown from seed treated with 4, 6 and 8 oz Benlate per 100 lbs seed were analyzed for the benomyl metabolites methyl 2-benzimidizolecarbamate (MBC) and 2-aminobenzimidizole (2-AB) by High Performance Liquid Chromatography (HPLC). Residue procedures and results will be reported.**

Microorganisms which have been associated with damping-off problems in seedlings were isolated from 'Florida Sweet' corn seedlings which later damped-off (1). Treatment of seeds with fungicides gave improved stands of 'Florida Sweet' corn and thereby increased yields (4). This report deals with residues resulting from seed treatment by one of these fungicides, benomyl [methyl 1-(butylcarbamoyl)-2-benzimidizolecarbamate], in kernels, cobs and husks of the mature plants.

Materials & Methods

'Florida Sweet' corn seeds treated with 4, 6 and 8 oz of 50% wettable Benlate powder per 100 lbs of seed were planted in peaty muck soil 0.5 ft apart in rows 3 ft apart in Belle Glade. At harvest, ears were husked, kernels were removed from cobs and husks, kernels and cob samples from the three Benlate treatments and a check were chopped in a Hobart food chopper and frozen until analysis.

50 g samples, including checks fortified with 0.5 and 2.5 ppm Benlate, of each of the treatments were extracted with 150 ml ethyl acetate by blending in a 1 qt Mason jar. The extraction and clean-up procedure involving dual solvent partitioning with pH adjustments is that of Pease and Gardiner (2). The method was modified to eliminate the boiling in caustic step after the hexane wash to avoid conversion of the methyl 2-benzimidazole carbamate (MBC) to 2-aminobenzimidazole (2-AB).

Quantitation of Benlate/MBC after extraction was conducted by high performance liquid chromatography. The LC instrument was equipped with a 1 meter glass column containing ETH and was operated under the following conditions: column temp. 35°-39°C; mobile phase, 10% methanol: 90% H_2O; carrier flow rate, 0.6 ml/min; inlet pressure, 600 Psi; UV detector, 280 nm, 0.01 absorbance full scale; recorder chart speed, 8 in/min. A 50% ethanol: 50% H_2O solution was used in the reference cell of the UV optical unit.

Sample and standards were injected as 2 μl aliquots. All residues were quantitated as MBC.

The fortified checks were quantitated by concentrating portions of the original spike solutions and generating a linear standard curve which was used to calculate percentage recovery. In addition, this fortified check curve was compared to a standard curve of MBC analytical standards to confirm the actual content of the spike solution.

The extracts of corn grown from treated seed and the untreated checks were injected and compared with injections of standard solutions of a combination of MBC and 2AB. In cases where an interfering peak appeared close to MBC, an aliquot of 7.5 μg of MBC was drawn up and injected with the sample to verify the separation of the peak from MBC.

Results & Discussion

Table 1 illustrates recoveries of Benlate from fortified check samples. The increase of recovery from lower to higher fortification levels is normal and although a higher than 50% recovery and less variability at the 0.1 ppm is desirable these recoveries were adjudged satisfactory. No Benlate, quantitated as MBC and 2-AB, was found in any of the samples.

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Table 1. Percentage recovery of Benlate (analyzed as MBC) from corn cobs, kernels and husks fortified with 2.5, 0.5 and 0.1 ppm Benlate.

	Cobs			Husks			Kernels		
	2.5	0.5	0.1	2.5	0.5	0.1	2.5	0.5	0.1
1.	112.0	64.0	—[z]	85.2	70.8	64.0	83.8	80.0	80.0
2.	115.0	102.0	50.0	87.2	62.0	20.0	87.4	48.0	124.0
3.	88.0	80.0	—[z]	34.8	82.0	140.0	81.4	76.8	120.0
4.	80.0	54.0		61.1	73.2	00.0	83.8	57.6	50.0
5.[x]	128.0	140.0		121.4	140.0		53.4	58.0	50.0
6.	57.6	67.2		98.6	44.0		76.0	49.6	
7	55.2	47.2		71.7	67.2		79.8	60.0	
8.	72.0	59.0		45.6	44.8		60.0	46.8	
9.	63.9	45.3		77.6	53.0		72.4	60.0	
10.[x]	89.6	40.0		98.9	56.8		97.4	60.0	
Total Recovery	86.1	69.9	50.0	78.3	69.4	56.0	77.5	59.7	87.0

[z]Samples inadvertently evaporated to dryness prior to injection.
[x]Samples stored at 0°C for 3 months after fortification prior to analysis.

If the amount of Benlate applied to the seed is taken into consideration one would expect very little to be present in the seeds of the mature plant. It has been shown, however, that Benlate is absorbed and translocated in corn tissue (3) so the possibility, however remote, of tissue residues in sweet corn did exist.

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EFFECT OF GROWTH REGULATORS ON STORAGE LIFE OF ONION SEED[1]

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Abstract. **The objective of this research was to improve the storage life of onion seed with growth regulators. Seed of 3 onion cultivars were treated with two concn of abscisic acid (ABA), gibberellic acid (GA), or kinetin (Kn) dissolved in dichloromethane (DCM). The seeds were stored in sealed jars at 2 temp (10 and 35°C) and at 2 seed moisture levels (10 and 20%). Germination percentage, rate, and ATP content were measured at 0, 3, and 9 months. GA and Kn had little effect on viability of 'Fiesta' and 'Premier' under any storage condition. However, germination in 'Elite', which lost the most viability and vigor, was improved during storage by treatment with Kn. Abscisic acid lowered germination and vigor in all cultivars. Onion seed lost viability and vigor quickly under adverse storage conditions of high temp and moisture. With optimum conditions, increased storage time from 3 to 9 months had no effect on onion seed germination and vigor. Seed ATP was higher in seed treated with growth regulators and did not always relate to germination and other vigor measurements.**

Onion seed has been regarded as having a short storage life, generally from 6 to 24 months under normal conditions (9). Rocha (15) found that onion seeds with 13 to 15% moisture were nonviable after 3.5 months storage at 21°C. However, seeds with moisture contents of 6.5 to 9.2% lost no viability after 7.5 months of sealed storage.

Growth regulators have an effect on seed germination. Abscisic acid (ABA) is one of the principal inhibitors involved in seed and bud dormancy in many species (2, 17, 18). Gibberellic acid (GA) promotes seed germination in a number of species. GA stimulates *de novo* synthesis of α-amylase in barley aleurone layers, a prerequisite to complete starch breakdown and germination (12). GA can substitute for red light to overcome dormancy in lettuce (6). Cytokinins are believed to promote seed germination when certain growth inhibitors are present (7). Kinetin (Kn), a synthetic cytokinin, overcame thermodormancy in lettuce seed (14). Kn pretreatment improved germination of new and old lettuce seed at 30°C (10).

Organic solvents, such as dichloromethane (DCM), have been used to incorporate chemicals into seeds without initiating germination. Meyer and Mayer (11) showed that the growth inhibitor, coumarin, can be applied to lettuce seeds with organic solvents, such as DCM. They proposed that the solvent helped the chemical penetrate deeply into the seed. Light-requiring lettuce seeds, treated with GA dissolved in acetone or DCM, had nearly 100% germination in the dark even after prolonged storage, and those treated with ABA failed to germinate at all (8). Several chemicals, such as fungicides, insecticides, and antibiotics, were applied to dry seeds of various species via organic solvents and preserved seed quality as determined by the germinating capability of the seed or ATP content (20).

The experiment that follows was designed to determine the effect of the growth regulators Kn, GA, and ABA on extending the storage life of onion seed stored under various conditions for 9 months.

Materials and Methods

Onion seeds (*Allium cepa L.* cvs. Fiesta, Elite, and Premier) were obtained from Stokes Seeds Ltd. The seed was produced in 1974 and was not treated with pesticides.

Seeds were soaked for one hour in dichloromethane containing abscisic acid (.25 and .5 mM), gibberellic acid (.5 and 1.0 mM), or kinetin (.5 and 1.0 mM). The seeds were then drained and dried in an oven at 35°C for one hour. Weighed amounts of seed were placed in small mason jars and seed moisture contents were equilibrated to 10 and 20% (15). Duplicated treatments were placed in incubators at either 10 or 35°C. Treatments were sampled at 0, 3, and 9 months for ATP content, germination percent, and germination rate.

ATP content was determined on 25 seeds from each sample according to the procedure of Ching and Danielson (3). All samples were imbibed with distilled water for 4 hours, then crushed and extracted with 10 ml of boiling distilled water for 10 minutes. After cooling, 5 ml of extract were diluted with 5 ml of buffer containing 0.05 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) and 0.05 M magnesium acetate.

The ATP assay was previously described (19). Freeze-dried firefly extract containing luciferin-luciferase was reconstituted by adding 5 ml of ice-cold distilled water which resulted in an enzyme preparation containing 0.05 M potassium arsenate and 0.02 M magnesium sulfate, pH 7.4. Light

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