

Microwave Treatment of Tobacco Seed to Eliminate Bacteria on the Seed Surface

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ABSTRACT

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Treatment with microwave radiation for 20 min eliminated *Erwinia carotovora* var. *carotovora* on infested tobacco seed coats without affecting germination.

Additional key words: microwave radiation, hollow stalk of tobacco.

Treating seeds by heating, or by washing, or coating with antibacterial or anti-fungal compounds has been used to eliminate microbial plant pathogens (1, 10). Some of the chemical treatments are no longer registered for use, and the physical treatments may not be satisfactory due to the time involved for treatment or lowered percentage of germination.

Recently we have found that *Erwinia carotovora* var. *carotovora*, the causal agent of hollow stalk of tobacco, may be transmitted via infested seed (J. McIntyre, D. C. Sands, and G. Taylor, *unpublished*). Soaking tobacco seed in water at 50 C for 12 to 18 min inactivated the organism without reducing germination. This treatment also has been used to eradicate inocula from the interior as well as the surface of other types of seed (10).

In this report we show that microwave radiation may be used to eliminate *E. carotovora* var. *carotovora*, the causal agent of hollow stalk of tobacco, from the surface of tobacco seed.

MATERIALS AND METHODS

Tobacco seed used was *Nicotiana tabacum* 'Consolidated L.' (ConL). The microwave source was a General Electric Model Jet 90 Microwave Oven (General Electric, Louisville, KY 40225) which was rated to deliver 625 W (high power) at a wavelength of 2,450 MHz.

Inoculum of *E. carotovora* var. *carotovora* was produced on a medium composed of mineral salts (6), 1.5% glycerol, and 1.1% agar (Noble agar, Difco, Detroit, MI 48232). After incubation for 24 hr at 30 C, bacterial cells were washed from the surface of the agar and suspended in water prior to coating the seeds. Seeds, infested by a 1-min immersion in a suspension of bacteria that contained 10^9 cells/ml, were air dried on paper for 3 hr.

Infested seeds (200-300, one layer deep) on a 15.5 cm diameter paper plate then were placed 2 cm above the floor in the center of the oven for treatment. A paper plate was used since glass containers became heated and plastic

containers softened and collapsed after about 5 min. After treatment, some of the seeds were tested for germination on blotting paper by a standard method (3), and the remainder placed on the surface of a selective medium for *Erwinia* spp. (5). Plates were incubated anaerobically (GasPak, Baltimore Biological Laboratories, Div. of Bioquest, Cockeysville, MD 21030) at 30 C and observed after 48 hr for presence of the test bacterium around each seed. Moisture content of seeds was determined by the AOAC method (2).

RESULTS

Treatment of tobacco seeds with 625 watts microwave radiation for 20 min eliminated viable *E. carotovora* var. *carotovora* without reducing seed germination (Fig. 1). The percentage of infested seed was reduced an average of 68% by a 10-min treatment and 99% by a 15-min treatment. Infested tobacco seed contained 5.3% moisture.

We also treated 10,000 seeds with 625 watts of microwave radiation for 20 min and examined them in the germinating chamber for the presence of chlorophyllless mutants. No such mutants were seen but questionable seedlings were planted in soil for further observation. None of these lacked chlorophyll.

Since it took 20 min to destroy bacteria on the surface of tobacco seed, we tested whether other seeds could withstand this length of microwave treatment and still remain viable. We found in three trials that germinability of bean seed was completely destroyed by a 2-min treatment and germinability of cabbage seed (four trials) was reduced 10% by a 2-min treatment but 55% by a 5-min treatment. The moisture content of the bean and cabbage seed was 8.2 and 4.8%, respectively.

DISCUSSION

For the three cultivars of seed that we tested there appears to be a relationship between size of seed and its ability to withstand microwave exposure and remain viable. The larger seeds did not tolerate long exposure

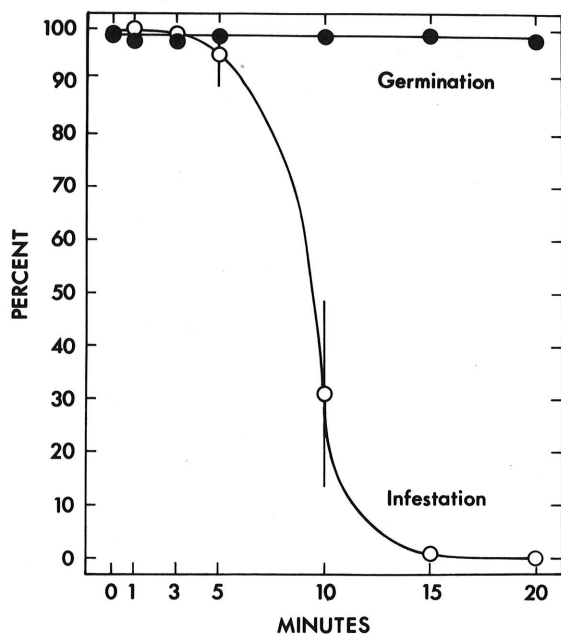


Fig. 1. Percent germination and decrease in percent of infestation of tobacco seed coated with *Erwinia carotovora* var. *carotovora* after treatment with microwave radiation at 625 watts at 2,450 MHz. Vertical lines indicate standard deviation (four trials for germination and three trials for infestation studies).

time. Since the water content of tobacco and cabbage were essentially similar, it is not this factor alone which determines resistance to microwave treatment. It is possible that larger seeds cannot radiate heat away from the seed during the microwave radiation.

Exactly how microorganisms are killed by microwaves is still uncertain. It is generally accepted that heat alone

kills the microorganisms, although it has been suggested that microwaves may somehow disrupt the microbial cells directly (4).

To the best of our knowledge this is the first report on treatment of small seeds by microwave radiation to destroy pathogenic microorganisms on the seed surface, although other types of radiation have been tested (7, 8, 9). Microwave treatment, for some types of seed, offers an alternative method to destroy pathogenic microorganisms on seeds.

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