Methyl Bromide and Steam Treatment of an Organic Soil for Control of Fusarium Yellows of Celery

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ABSTRACT

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Fumigation of an organic soil naturally infested with *Fusarium oxysporum* f. sp. *apii* race 2 with a mixture of 98% methyl bromide and 2% chloropicrin (0.15 kg a.i./m^2) eradicated the pathogen that causes Fusarium yellows of celery from both the soil and the celery root pieces in the soil. Celery seedlings and transplants grown for 12 and 5 wk, respectively, in the fumigated soil were disease-free. Pasteurization (70 C, 30 min) and autoclaving (121 C, 3 hr) of the soil also destroyed the pathogen and, in addition, greatly stimulated growth of celery seedlings. In general, top growth of direct-seeded celery was not affected by duration of poststeaming soil incubation. When celery was transplanted into unincubated steamed soils, however, considerable injury occurred and top growth was significantly less than in soils incubated for 12, 24, or 36 days.

Several measures aimed at controlling Fusarium yellows of celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.) caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *apii* (R.R. Nelson & Sherb.) W.C. Snyder & H.N. Hans. race 2 have been tried in the past with discouraging results (3). A promising control may be the prevention of the pathogen establishment in disease-free fields. Awuah et al (3) determined that the use of celery transplants grown in seedbeds (organic soil) naturally infested with *F. o. apii* race 2 was most responsible for the rapid spread of the disease in New York. Thus, eradication of the pathogen from seedbeds must be the first consideration in preventing its spread.

Even though methyl bromide has been successfully used for controlling fungal pathogens in mineral soils (8,10,12), its efficacy on organic soils has not been adequately demonstrated. Similarly, while steam treatment of mineral soils is commonplace, the utility of the practice on natural organic soils is not well known. This paper reports a laboratory and glasshouse study on fumigating, autoclaving, and pasteurizing an organic soil to eradicate *F. o. apii* race 2.

MATERIALS AND METHODS

Source of organic soil. The natural organic soil utilized in the study was obtained during the late summer from a commercial celery field in Orange County, New York, just before harvest. Because three successive crops of celery had been grown in the field and most plants in the third year of cropping were infected with the pathogen, the soil was infested naturally with a high level of propagules of *F. o. apii* race 2. The soil sampled was a Carisle muck, which is a mesic, euic, medisapric (typic) histosol containing greater than 50% organic matter with a bulk density of 0.5 g/cm^3 and an average pH of 6.1.

Soil disinfestation. The soil was prepared and then fumigated with a mixture of methyl bromide and chlorpicrin (98% methyl bromide, 2% chloropicrin as a warning gas). The soil first was spread on a polyethylene tarpaulin in a 9.3 m^2 (100 ft^2) area to a depth of 10.2–15.2 cm. The soil then was covered with another polyethylene tarpaulin, and 1.36 kg (3 lb) of the fumigant was volatilized over the soil under the polyethylene cover. During the time of fumigation, the soil temperature ranged from 10 to 13 C and the moisture level of the organic soil was normal for good plant growth (60% H$_2$O on a volume basis that is neither too wet nor too dry for an organic soil). After 24 hr, the fumigated soil was uncovered, aerated, and stored in 10 clean plastic containers; the container tops were left on for 1 wk, then removed. During the first week and for an additional 4 wk of storage, the soil was maintained in an unheated pole barn at room temperature before being used.

A 10-kg sample of the soil in an open metal container (metal bushel basket) was treated for 3 hr at 121 C and 124.02 kPa (18 psi) in an autoclave (American Sterilizer Company, Erie, PA). The autoclaved soil then was stored uncovered for 2 wk in the same container in the glasshouse before being used.

The natural field soil also was treated with a soil pasteurizer (Fig. 1) (1). Typically, a 10-kg sample was treated at 70 C for 30 min, then placed and stored in an open metal bushel basket for 2 wk in the glasshouse before being used.

Assay of fumigated and autoclaved soils. Representative soil samples were taken from the fumigated soil (one from each of the 10 buckets at 5 wk after fumigation) as well as from the autoclaved soil. The samples were assayed for *F. o. apii* race 2 and other fungi on a potato-dextrose agar amended with 600 mg/L of chloramphenicol (CPDA) (2). At the same time, soil samples were distributed into four attached cells from a Foster Grant plastic insert (insert No. 3601, Syracuse Pottery, Inc., Syracuse, NY). Seeds of the celery cultivar Florida 683 were planted in the soil in each cell. The resulting seedlings were grown for 5 wk, then examined for damping-off, root decay, and vigor. In a further bioassay, 3-wk-old Florida 683 plants were transplanted into plastic pots (12.5 cm [5 in.] diameter), one plant per pot, containing treated soils. Incidence of Fusarium yellows was recorded after 12 wk. Isolation of *F. o. apii* race 2 from celery root pieces in both autoclaved and fumigated soils was attempted (3). In all these assays, the natural organic soil (untreated) served as the control.

Effect of poststeaming incubation period. Samples (10 kg) of the natural organic soil were either pasteurized or autoclaved for 30 min. After 0, 12, 24, or 36 days of incubation, soil samples were distributed into plastic cups (250 ml) and 4-wk-old Florida 683 plants were transplanted at the rate of one per cup. After the plants had grown under glasshouse conditions for 2 wk, fresh and dry weights of the tops were determined. Additionally, the treated soils were distributed into the plastic growing inserts previously described and direct-seeded with Florida 683 (100 mg seeds per cell). Seedlings were grown under glasshouse conditions for 4 wk, at which time fresh and dry weights of tops were determined (20 seedlings from each of four replications in each treatment). Populations of fungi colonizing treated soils during incubation were determined using either a sorbose medium (20 g of L-sorbose, 20 g of Bacto agar, 600 mg of chloramphenicol, 1 L of distilled water) or CPDA (2).

Plant growing conditions. All plants were grown in a glasshouse at 27–29 C

with a 16-hr photoperiod provided by supplemental cool-white fluorescent light tubes. The plants were watered equally when necessary.

RESULTS

Fumigation with methyl bromide eliminated *F. o. apii* race 2 from the natural organic soil and celery root pieces present in the soil. No isolates of *Fusarium* spp. were recovered from any of the celery root pieces in this treatment, and other fungi (*Penicillium* spp. and *Alternaria* spp.) were recovered from only 29% of the root pieces (Table 1). Mucoraceous and pythiaceous fungi as well as weeds were also destroyed. Autoclaving the soil eliminated all fungi from the soil and from the celery root pieces in the soil.

Although celery transplants grown in the natural field soil developed Fusarium yellows after 12 wk, those grown in the fumigated and autoclaved soils were disease-free. Direct-seeded celery in the treated soils also were disease-free,

whereas seedlings in the untreated control soil were damped-off and roots of surviving seedlings were mostly decayed after 5 wk. Four-week-old celery seedlings transplanted into the unincubated autoclaved and pasteurized soils showed symptoms of injury, and top growth was less than in soils incubated for 12, 24, or 36 days (Fig. 2A). No injury was observed in direct-seeded celery, however, and top growth of the seedlings generally was not affected by duration of soil incubation (Fig. 2B).

Fungal recolonization of autoclaved soils was greater than that of pasteurized soil. In the former, total fungal propagules increased from 0 to 135.5×10^4 propagules per gram of soil after 36 days (Fig. 3). The fungal population in the pasteurized soil was 0.1×10^4 propagules per gram of soil shortly after pasteurization and 0.5×10^4 propagules per gram of soil after 36 days (Fig. 3). *Aspergillus* spp. and *Penicillium* spp. were the dominant colonizers.

DISCUSSION

The effectiveness of methyl bromide in controlling nematodes, weeds, bacteria, and fungi in mineral soils is well known (4,8,10,12). Elimination of propagules of *F. o. apii* race 2 from the organic soil utilized in the present study is consistent with the broad-spectrum efficacy of methyl bromide. The fumigant also was able to penetrate infected celery root pieces in the organic soil and destroy the pathogen. Stark and Lear (12) demonstrated that methyl bromide could penetrate root-knot galls and kill the embedded nematodes. Destruction of sclerotia of *Botrytis cinerea* Pers.:Fr. (8), *Sclerotinia sclerotiorum* (Lib.) de Bary (9), and *Sclerotium delphinii* Welch (8) with methyl bromide in mineral soils indicates the high penetrability of the fumigant into hard fungal tissue. Thus, eradication of *F. o. apii* race 2 from celery root pieces, though somewhat surprising, is not without precedent. The potential utility of this fumigant for eradicating
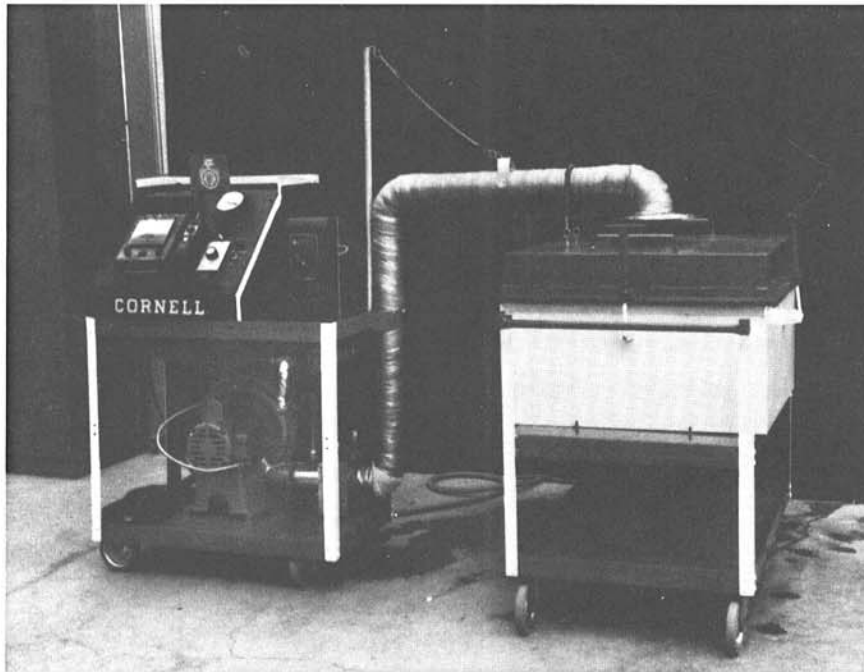

Fig. 1. Equipment used for pasteurizing soil with aerated steam.

Table 1. Isolation of fungi from celery root pieces extracted from an organic soil fumigated with methyl bromide or autoclaved[a]

Root pieces with fungi[b] (%)	Soil treatment		
	Fumigated	Autoclaved	Untreated[c]
Fusarium oxysporum f. sp. *apii* race 2	0	0	45
Other *Fusarium* spp.	0	0	42
Penicillium spp.	17	0	0
Alternaria spp.	12	0	0
Trichoderma spp.	0	0	7
No fungal growth	71	100	4

[a] Soil was autoclaved for 3 hr at 121 C, then assayed immediately. Fumigated soil (98% methyl bromide and 2% chloropicrin as a warning gas, 0.15 kg a.i./m²) was assayed 5 wk after treatment.
[b] Percentage of root pieces from which fungi grew.
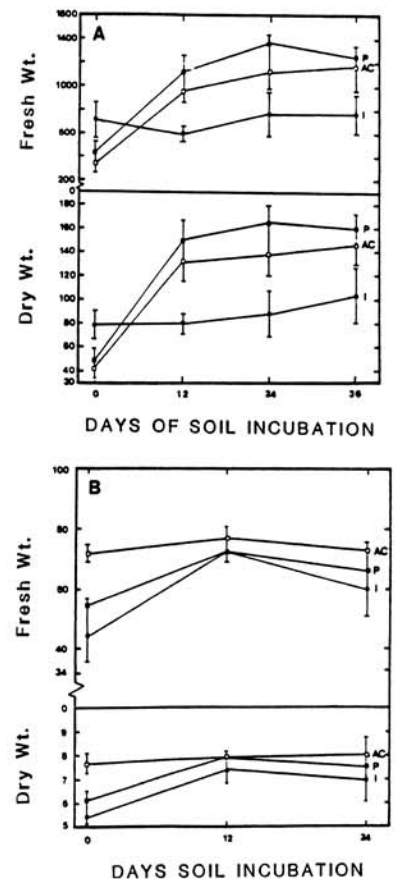[c] Natural field soil.

Fig. 2. Top growth (milligrams) of celery plants in autoclaved and pasteurized organic soils at different durations of poststeaming incubation after (A) transplanting and (B) direct seeding into the treated soils. Each data point in A is the mean of seven replications, and each data point in B (representing the mean weight per seedling) is the mean of four replications (20 seedlings per replication). Vertical bars indicate an interval that is twice the standard error of the mean. AC = autoclaved soil, P = pasteurized soil, I = untreated soil.

Fig. 3. Colonization of autoclaved, pasteurized, and untreated soil by fungi as determined by dilution plating on potato-dextrose agar amended with chloramphenicol. Each data point is the mean of four replications. Vertical bars indicate an interval that is twice the standard error of the mean. AC = autoclaved soil, P = pasteurized soil, I = untreated soil.
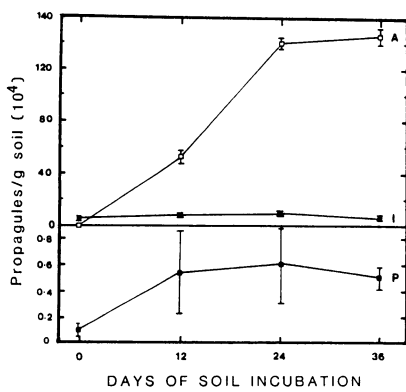
F. o. apii race 2 from naturally infested soil used in celery seedbeds is now established. Methyl bromide fumigation of organic soils should help prevent spread of the pathogen by the production of disease-free celery transplants.

In the fumigation experiment, a relatively high rate of methyl bromide was used for a thin layer of soil. This was because the prevailing weather (10–13 C) was cooler than desired for soil fumigation. Of more importance, however, it was believed that because fumigation involved an organic soil, a high rate of the fumigant was necessary for a high level of pathogen destruction (14). Eradication of *F. o. apii* was demonstrated in an artificial system, but the maximum depth to which the fumigant would be effective in the organic soil remains to be determined. However, in the interim period since our research was accomplished and the present, and on the basis of our research results, one New York celery grower has adopted the practice of an annual methyl bromide fumigation of all of his celery seedbeds (soil in seed beds on glasshouse benches or in outside seedbeds adjacent to the glasshouses). He has yet to have any plants infected with *F. o. apii* race 2 in the fumigated seedbeds, and apparently he has eliminated this potential source of the pathogen for

introduction into his celery production fields.

Treatment with methyl bromide of severely and extensively infested celery fields, though possible, is not economical. However, treatment of specific areas of infestation with the fumigant might be feasible. Spot fumigation in the field would have to be effected in an integrated manner with the use of disease-free transplants for the practice to be successful. For a valuable vegetable such as celery, field treatment with methyl bromide on a limited scale might be feasible.

Pasteurizing and autoclaving the natural organic soil utilized in our study eradicated *F. o. apii* race 2 and resulted in better growth of celery plants than in untreated soil. While attributing the vigorous growth of celery plants in steamed soil to destruction of the pathogen, we recognize that released nutrients may contribute to the observed growth response, as has been demonstrated for lettuce (5). Further experiments are required to confirm this possibility.

Although at least 12 days of soil incubation were necessary in the case of transplanted celery, the crop could be direct-seeded on the steamed soils without any soil incubation. With the latter method of plant establishment, soil toxicity after steaming (11,15) could be reduced significantly (by leaching during watering) before seed germination. Thus, the resulting seedlings would not be exposed to the injurious levels of NH_4^{++}, Mn^{++}, and soluble salts that are generally blamed for poststeaming soil toxicity (6,13). Lack of soil incubation before direct seeding with celery could be an added advantage if commercial seedbeds containing organic soil are steamed for the purpose of destroying *F. o. apii* race 2.

The faster recolonization by *Aspergillus* spp., *Penicillium* spp., and other fungi in autoclaved soil than in pasteurized soil most likely was due to the microbial buffering capacity of the pasteurized soil. Because of the experimental design, we suspect that the establishment of *Penicillium* spp. and *Aspergillus* spp., the dominant fungi in the steamed soils, probably resulted from reinfestation by airborne conidia of these fungi. Microbial recolonization of

steamed soil also has been reported by Martin (7). Thus, there is always the possibility that infection of celery seedlings could occur in seedbeds treated with steam if the necessary precautions are not taken to prevent recontamination of the steamed soil with *F. o. apii* race 2.

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