

Effect of Biocidal Treatments on Cation Exchange Capacity and Fusarium Blight of Soybean in Delaware Soils

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ABSTRACT

Fusarium wilt has caused significant losses in soybean [*Glycine max* (L.) Merr.] production and is very difficult to control because it is caused by the soil-borne fungus *Fusarium oxysporum*. A better understanding of the relationship between soil characteristics and the pathogen and between biocidal treatments and physiochemical properties is needed, especially for soils that appear to be *suppressive*. The effects of autoclaving and Vapam fumigation on cation exchange capacity, organic matter content, clay content, pH, and Fusarium wilt of soybean were determined for several different Delaware soil types (Typic Hapludults, Typic Quartzipsamments, and Typic Umbraquults). 'Essex' soybean was grown in the treated soils that had been infested, prior to planting, with 6.3×10^4 viable spores per gram of dry soil of a pathogenic *Fusarium oxysporum* isolate. Treatments were arranged on the greenhouse bench in a randomized complete-block design with five replications. Cation exchange capacity generally increased with fumigation and decreased with autoclaving, but differences were not significant for all soils. Organic matter content increased following fumigation in three soils, and pH values were lowered by both biocidal treatments in all soils except a Matapeake silt loam. Disease incidence and severity were affected differently for each soil type.

Additional index words: *Glycine max* (L.) Merr., *Fusarium oxysporum*, Autoclaving, Vapam fumigation, *Suppressive soil*.

VARIOUS races of *Fusarium oxysporum* Schlecht. emend. Snyder and Hans. cause a wilt disease of soybean that reduces yield (Cromwell, 1917; Armstrong and Armstrong, 1965; Ross, 1965). Since 1976,

serious losses from the disease have occurred on the Delmarva (Delaware, Maryland, and Virginia) peninsula, and losses as high as 59% of normal yield have been reported (Ferrant and Carroll, 1981; Leath and Carroll, 1982, 1985). Subsequent studies have shown that the pathogen reduces nodule number and the N_2 -fixing capacity of infected plants (Corriveau and Carroll, 1984), and that common dicotyledonous weeds in soybean fields can serve as symptomless hosts of the pathogen (Helbig and Carroll, 1984). The pathogen occurs in most soybean growing areas and the potential exists for widespread occurrence of the disease. With more than 99 225 ha of soybean cultivated in Delaware in 1983 (Smith, 1985), the necessity for adequate control of this fungal pathogen is imperative. The use of resistant cultivars is an important method

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Published in *Agron. J.* 80:8-12 (1988).

of disease control but requires extensive screening of lines, which may be time consuming (Leath and Carroll, 1982, 1985). In the past, application of fungicides to the soil has provided only limited control of soil-borne pathogens such as *Fusarium*. Thus, in the past two decades research has been directed toward discerning the characteristics of soils that have been shown to suppress disease incidence caused by certain soil-borne pathogens on particular crops.

Suppressive soils are defined as those soils in which disease development is suppressed even though the pathogen is introduced in the presence of a susceptible host (Baker and Cook, 1974). It has also been described as an *umbrella* term encompassing fungistasis, competitive saprophytic ability, and other disease and pathogen interactions in which the defined relationship of reduced disease in the presence of the pathogen and susceptible host exists (Huber and Schneider, 1982). A review of the literature by Schneider (1982) indicated the inherent complexities underlying the mechanisms and characteristics of suppressive soils. Researchers have initiated investigations into the role that soil physiochemical properties have on the phenomenon of soil suppressiveness. For example, Stotzky and Rem (1966) investigated the relationship of soil mineralogy to microbial respiration and found a positive correlation with cation exchange capacity (CEC). Burke et al. (1970, 1972) found that soil compaction is related to the occurrence of *Fusarium* root rot of bean (*Phaseolus vulgaris* L.). Recent studies on suppressive and conducive soils by Martin and Hancock (1986) showed no consistent relationship between suppressiveness and CEC. However, these researchers did not focus on biocidal treatments. Biocidal treatments such as autoclaving and fumigation have become important components of phytopathological studies focusing on the interactions of the soil, soil-borne pathogens, and the resultant disease losses. However, very little attention has been directed toward the effect these biocidal treatments may have on the physiochemical properties of the soils studied and how this may in turn affect suppressiveness.

The objectives of our study were to examine the effects of two common sterilization techniques, autoclaving and Vapam fumigation, on CEC, organic matter (OM) content, clay content, and pH of five Delaware soils, and determine if these treatments significantly affect the occurrence and severity of *Fusarium* wilt of soybean grown in the greenhouse. Some preliminary results were reported previously (Sandler et al., 1984).

MATERIALS AND METHODS

Soil Characterization and Analyses

Representative soils of Delaware, with varying physiochemical properties, were collected for this study: (a) a Matapeak silt loam (fine-silty, mixed, mesic Typic Hapludults) from Dover; (b) a Matapeake silt loam from Newark; (c) an Evesboro loamy sand (mesic, coated Typic Quartzipsamments) from Millsboro; (d) a Sassafras loamy sand (fine-loamy, siliceous, mesic Typic Hapludults) from Georgetown; and (e) a Pocomoke loamy sand (coarse-loamy, siliceous, thermic Typic Umbraquults) from Georgetown. Soils (a) and (c) were collected from virgin sites, whereas soils (b) and (d) were from sites that had been in *Fusarium* wilt-soybean ex-

periments for 3 and 10 yr, respectively. Results from previous studies with these soils indicated that the Matapeake-Newark soil was suppressive to *Fusarium* wilt whereas the Sassafras soil was *wilt-conducive* (Leath and Carroll, 1982, 1985). Soil (e) came from a site that had been planted with soybean for four consecutive years and had a history of *Fusarium* wilt occurrence. The clay contents of Soils (a) to (e), as determined by the pipet method (Day, 1965), were 11.0, 15.0, 5.4, 6.2, and 7.0%, respectively.

At each site, soil was randomly taken from the top 0.15 m, composited, and placed into large plastic bags. The soils were maintained at the field-extracted moisture content for 7 d (Burke, 1965). Approximately 45 kg of each soil type was divided into three equal portions, air-dried in the greenhouse, and passed through a 2-mm sieve in preparation for basic laboratory and greenhouse analyses.

A 10-cm-deep layer of the first portion of each sample was fumigated for 48 h with Vapam (sodium N-methyl dithiocarbamate) at the rate of 25 mL L⁻¹ per 0.84 m² of soil, utilizing a total volume of 2.5 L of solution. These portions were then aerated for 21 d before storage in large metal containers. The second portion of each sample was autoclaved at 394 K for 1 h and after 24 h, autoclaved again for 1 h. The portions were stored on the greenhouse bench in wooden flats and covered to prevent contamination. The third portion from each soil was left untreated and served as the control.

Following treatment, random samples were taken from each soil portion to determine pH, CEC, OM content, and clay content. The measurement of each of these variables was replicated four times.

The CEC of the soils was measured at pH values of 5, 6, and 7. To obtain a constant pH during the exchange reaction, the suspension was adjusted prior to these studies by successive washings with solutions at the appropriate pH, using NaOH or HCl. The washings were repeated until there was no pH change after 24 h of equilibration on a reciprocating shaker.

After pH adjustment, the suspensions were centrifuged and the CEC was determined by a MgCl₂ saturation with subsequent displacement with CaCl₂ at pH values of 5, 6, and 7 using procedures outlined by Rich (1962) and Okazaki et al. (1963). Magnesium was determined using atomic absorption spectrophotometry.

Organic matter was determined using the Walkley-Black method (Allison et al., 1965). The pH of each treated sample was measured in deionized water and in 0.01 M CaCl₂ using a 1:1 (w/v) ratio. The remainder of each soil portion was utilized as the greenhouse growth medium for the *Fusarium* inoculation studies.

Greenhouse Studies

Approximately 2.1 kg of each treated soil were placed into surface-disinfested 17.8-cm-diam plastic pots. These soils were inoculated by mixing in a mycelial and conidial suspension of a single-spored *F. oxysporum* isolate pathogenic to soybean. Inoculum was added at the rate of 6.3×10^4 viable propagules per gram of dry soil as determined by dilution plate counts. Pots with correspondingly treated soils without inoculum served as the controls. Pots were arranged on the greenhouse bench in a randomized complete-block design with five replicates. Seven soybean seeds of the cultivar Essex were then planted in each pot and thinned to five plants after seedling emergence. Essex soybean is highly susceptible to the test pathogen (Leath and Carroll, 1982, 1985).

After 9 wk, individual plants were removed from the pots and the roots were rinsed with water to remove soil, followed by excision at the first node. The following rating scale was used to assess disease of the root and hypocotyl tissue caused by *F. oxysporum*: 1 = healthy, no evidence of infection; 2

= slight external necrosis of basal stem and tap root; 3 = moderate lesion development on basal stem or roots, or both; 4 = extensive lesion development on basal stem and roots; and 5 = extensive lesion development on basal stem and roots with vascular discoloration. To ensure that *F. oxysporum* was responsible for the disease symptoms observed, isolations were made from diseased plants randomly selected from each soil treatment. One-centimeter sections of root and hypocotyl tissue were surface-disinfested with 7×10^5 mL of ethanol for 45 s and 1.05 kg m^{-3} of bleach for 1 min, followed by a rinse in sterile distilled water. Sections were then placed on acidified potato-dextrose agar and incubated at 301 K until resultant fungal colonies could be identified.

The data were analyzed using analyses of variance from the Statistical Analysis System (SAS Institute, 1982) and means were separated with the LSD at $P = 0.05$.

RESULTS AND DISCUSSION

Effect of Biocidal Treatments on Soil Chemical Properties

Table 1 shows the mean values for CEC at the three pH values for the soils subjected to the biocidal treatments. Fumigation significantly increased the CEC of the Evesboro soil at pH 5 and pH 6 when compared to both the autoclaved and untreated soils. Autoclaving decreased the CEC at pH 5 in the Matapeake-Dover soil. Both treatments increased the CEC in the Pocomoke at pH 5 whereas at pH 6, autoclaving decreased the CEC. The values obtained for the CEC of this soil are a reflection of its high OM content (Table 2).

Autoclaving significantly decreased the CEC at pH 5 in the Matapeake-Newark soil when compared to both fumigated and untreated soil. At pH 6 and pH 7 both treatments are similar to the untreated soil, but differed from each other (Table 1). A different pattern of treatment effect on CEC emerged with the Sassafras soil where autoclaving and fumigation both significantly decreased CEC at all pH values (Table 1). There was no difference between the effects of autoclaving and fumigation on the Sassafras soil at any pH level.

Table 1. Effect of biocidal treatments on the CEC of five Delaware soils at three pH values.

Treatment	Soil type				
	Evesboro loamy sand	Matapeake silt loam (Dover)	Pocomoke loamy sand	Matapeake silt loam (Newark)	Sassafras loamy sand
	pH 5.0				
Untreated	0.60†	4.20	3.26	5.52	2.90
Fumigated	1.77	4.97	6.10	5.26	1.19
Autoclaved	0.70	2.40	5.49	2.66	1.56
LSD (0.05)	0.77	1.74	0.98	1.88	0.55
	pH 6.0				
Untreated	1.20	5.56	11.14	5.64	3.10
Fumigated	1.90	6.10	11.84	6.00	1.50
Autoclaved	1.00	4.90	9.22	5.20	1.60
LSD (0.05)	0.60	NS‡	1.41	0.71	0.41
	pH 7.0				
Untreated	1.50	8.40	13.10	6.70	5.70
Fumigated	3.04	9.90	13.58	8.80	3.04
Autoclaved	1.32	6.91	14.50	5.57	3.16
LSD (0.05)	NS	1.76	NS	2.94	1.73

† Mean values expressed in cmol kg^{-1} .

‡ NS = no significant differences at the $P = 0.05$ level.

In general, the CEC did not change significantly when subjected to the biocidal treatments (nine of 15 cases for fumigation, eight of 15 cases for autoclaving). When changes were apparent, autoclaving decreased the CEC in six cases, compared to only one case of a CEC increase. Increases and decreases of CEC were equally distributed for the remaining soils subjected to fumigation. The high temperature at which autoclaving is carried out is sufficient to cause a change in CEC (Thomas, 1977; Way, 1850) by disrupting the OM and destroying adsorption sites. The results of this experiment are similar to those of Eno and Popenoe (1964), who found that fumigation and steam sterilization had no effect on CEC of a loamy fine sand and an Everglades muck soil.

Vapam is a non-volatile compound added to the soil in a water mixture. Once in the soil, it degrades into methyl isothiocyanate, which is relatively unstable and volatile (Goring, 1967). Under acid conditions, other by-products are formed including carbon disulfide, methyl amine, and elemental S (Turner et al., 1962). Goring (1967) suggested that methyl amine may break at its N-H linkages, and H bonding with the hydroxyl or carboxyl groups of the OM may occur. Perhaps this might be one mechanism that could account for the increase in adsorption sites in the soils where fumigation increased CEC.

We propose that the effect of biocidal treatments on CEC is related primarily to the OM content of the soil. Eno and Popenoe (1964) noted the importance of OM in their studies. Powlson (1975) stressed the influence of biocidal treatments on OM content.

The effect of biocidal treatments on CEC and OM for the Evesboro and Sassafras soils are shown in Tables 1 and 2. The fumigated Evesboro soil had a higher OM content than the untreated and autoclaved samples. The untreated Sassafras soil had a higher OM content than the fumigated or autoclaved Sassafras soil. These patterns can also be seen in the CEC values for these soils.

Organic matter content was greater in the fumigated Pocomoke, Evesboro, and Matapeake-Newark soils and lower in the Matapeake-Dover soil after fumigation. The reasons for these results are not clear, but may be related to the biocidal treatments which could have affected the degree and efficiency of oxidation of the organic materials. Eno and Popenoe (1964) found that steam sterilization increased OM content, whereas fumigation decreased it.

Autoclaving caused no change in OM content for either the Evesboro or Matapeake-Newark soils when compared to the untreated soil (Table 2). Overall, autoclaving had a variable effect on the OM of the soils studied. Powlson and Jenkinson (1976) found that autoclaving caused an increase in soluble OM. While autoclaving increased the OM content of the Pocomoke when compared to the untreated soil, fumigation caused the greatest increase for all but the Matapeake-Dover soil.

There was essentially no change in the percentage of clay in any of the samples following autoclaving. However, there were significant changes due to fumigation. For example, the percentage of clay was lowered from 5.4 to 2.8 and 11.0 to 9.0 for the Evesboro

Table 2. Effect of biocidal treatments on the OM content of five Delaware soils.

Treatment	Soil type				
	Evesboro loamy sand	Matapeake silt loam (Dover)	Pocomoke loamy sand	Matapeake silt loam (Newark)	Sassafras loamy sand
	g kg ⁻¹				
Untreated	10.0	45.0	113.0	15.0	28.0
Fumigated	14.0	42.5	150.0	20.0	25.0
Autoclaved	11.0	43.8	142.0	17.0	24.0
LSD (0.05)	1.8	1.1	6.8	2.2	3.0

and Matapeake-Dover soils, respectively. For the Matapeake-Newark soil, the clay content increased from 15 to 19%. This change should not be viewed as a methodology phenomenon since the pipet method used in this study is the most precise way to measure particle size analyses.

The pH values measured for the soils are listed in Table 3. Except for the Matapeake-Dover soil, treated soils had lower pH values than untreated soils. Except for the Matapeake-Dover and Evesboro (CaCl₂) soils, fumigated soils had the lowest readings as measured in both water and 0.01 M CaCl₂. Skipper and Westermann (1973) found similar results and attributed the decrease in pH to the release of organic acids brought on by the biocidal treatments.

Effect of Biocidal Treatment on Disease Severity and Incidence

Autoclaving and fumigation significantly decreased disease severity on the soybean planted in two of the three uninoculated soils that had been in cultivation with soybean for several years with a history of Fusarium wilt occurrence (Pocomoke and Matapeake-Newark, Table 4). These effects might be due to the reduction of the existing populations of Fusarium in the soils. The Evesboro and Matapeake-Dover soils had never been cultivated nor associated with populations of pathogenic *F. oxysporum*, and showed no evidence of disease development and hence no disease reduction due to biocidal treatments. The soybean in the treated Sassafras soil showed no differences with respect to disease severity.

Table 3. pH values of five Delaware soils subjected to biocidal treatments.

Treatment	Soil type				
	Evesboro loamy sand	Matapeake silt loam (Dover)	Pocomoke loamy sand	Matapeake silt loam (Newark)	Sassafras loamy sand
	pH in H ₂ O				
Untreated	4.6	5.2	5.2	6.5	5.7
Fumigated	3.0	5.2	4.6	6.0	5.2
Autoclaved	4.1	5.5	4.8	6.3	5.4
	pH in 0.01 M CaCl ₂				
Untreated	4.3	4.8	4.9	6.0	5.2
Fumigated	4.0	4.9	4.0	5.8	4.8
Autoclaved	3.9	5.2	4.4	5.9	4.9

Disease severity on the soybean in the inoculated soils was greater than that in the uninoculated soils for all treatments in the greenhouse test (Table 4). Autoclaving decreased the severity of Fusarium wilt in the Matapeake-Newark and Sassafras soils. The opposite was true, however, for the Evesboro soil where autoclaving resulted in a significantly higher disease rating. The pattern of biocidal treatments increasing disease severity has been documented in the literature (Baker, 1981; Mishra and Pandey, 1978). A decrease in severity with biocidal treatments is contrary to most other studies on suppressive soils (Schneider, 1982). The Sassafras soil shows a corresponding decrease in CEC and OM content upon autoclaving (Tables 1 and 2). For the Sassafras soil, chemical properties of the soil changed with biocidal treatments, with a subsequent decrease in disease severity.

Disease incidence was significantly decreased by both autoclaving and fumigation for soybean grown in the uninoculated Pocomoke and Matapeake-Newark soils (Table 4). Disease incidence correlated well with disease severity with respect to effects of the biocidal treatments. Fumigation has been shown to improve plant growth by increasing OM and microbial activity (Powelson and Jenkinson, 1976). The Sassafras soil showed a relationship between the biocidal treatments, changes in CEC, OM content, and disease severity of inoculated soybean.

Autoclaving and fumigation both increased disease

Table 4. Comparison of disease incidence and severity on soybean grown in uninoculated and inoculated soils subjected to biocidal treatments.

Treatment	Evesboro loamy sand		Matapeake silt loam (Dover)		Pocomoke loamy sand		Matapeake silt loam (Newark)		Sassafras loamy sand	
	Incidence†	Severity‡	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
	Uninoculated									
Untreated	0.2	1.04	0.4	1.16	4.2	2.84	4.6	2.64	3.8	2.56
Fumigated	0.2	1.00	0.4	1.08	2.4	2.08	0.6	1.12	1.8	1.56
Autoclaved	1.2	1.24	0.6	1.08	0.4	1.08	0.8	1.20	1.4	1.52
LSD (0.05)	NS§	NS	NS	NS	1.6	1.11	0.8	0.52	NS	NS
	Inoculated#									
Untreated	2.2	1.92	0.4	1.28	5.0	4.28	5.0	3.64	5.0	4.04
Fumigated	5.0	3.16	2.4	1.72	4.4	3.24	3.4	2.40	4.6	3.88
Autoclaved	4.6	3.52	1.0	1.20	4.6	3.76	2.4	1.92	3.8	2.72
LSD (0.05)	0.9	0.94	1.9	NS	NS	NS	2.4	1.49	NS	0.97

† Mean of five replications (five plants/rep.) rated on a scale from 0 to 5 where 0 = no plants in a pot infected and 5 = all five plants infected.

‡ Mean of five replications (five plants/rep.) where disease was rated on a scale from 1 to 5 where 1 = healthy, no evidence of infection and 5 = extensive lesion development on basal stem and roots with vascular discoloration.

§ NS = no significant differences at the $P = 0.05$ level.

Inoculated with a pathogenic isolate of *Fusarium oxysporum*.

incidence for soybean in the inoculated Evesboro soil (Table 4). This is an example of the increase that has been noted in literature presently available (Schneider, 1982). For the Matapeake-Newark soil, autoclaving significantly decreased disease incidence. There is no apparent relationship among changes in CEC, OM content, and the occurrence of disease in this treatment. Fumigation increased disease incidence in the inoculated Evesboro and Matapeake-Dover soils, but had no effect in the other soils.

In all experiments, isolations made from the randomly selected diseased plants yielded cultures of *F. oxysporum* that appeared to be identical to the culture used for inoculations. This confirmed that the test pathogen was responsible for the disease symptoms obtained in the study.

CONCLUSIONS

Biocidal treatments did not consistently affect CEC. However, in certain soils, i.e., Sassafras, the treatments significantly reduced the CEC. Organic matter content, which increased by fumigation with Vapam in four of the five soils tested, seems to be an important component linking the chemical and microbial fractions of the soil. A change in OM is likely to affect either or both of these factors. This change could significantly influence the incidence or severity, or both, of a particular disease. The wilt-conductive Sassafras soil, with a history of *Fusarium* wilt, illustrates the effect biocidal treatments can have on changes in the chemical properties of soils and subsequent disease development. However, this study involved only a single pathogen isolate and soybean cultivar. Soil-plant-pathogen situations are unique and must be considered individually in order to accurately describe the interactions and properties involved. Evidence gathered in this experiment supports the hypothesis that researchers should consider the effect of biocidal treatments on the chemical properties of soils and how these in turn influence the phenomenon of soil suppressiveness and disease occurrence.

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