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USE OF KEY INDICATOR PROCESSES TO ASSESS THE EFFECTS OF FUNGICIDES ON SOIL MICROBIAL PROCESSES AND NITROGEN DYNAMICS

DISSERTATION

Presented in Partial Fulfillment of the Requirement for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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*****

The Ohio State University
1997

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ABSTRACT

Harmonized methods are necessary to evaluate accurately the impact of pesticides on soil microorganisms, which play an essential role in soil biological processes including nitrogen dynamics, organic matter decomposition and nutrient availability. Although a number of studies on the effects of pesticides, mainly herbicides and insecticides, on soil microorganisms have been reported, very little research has been done to examine the long-term effects of fungicides on soil microbial activities and ecological processes, which are important in maintaining soil fertility. In addition, the impact of fungicides on soil processes, based on interrelationships within microbial populations and community- or system-level interactions, are also poorly understood. In my research, three broad-spectrum fungicides: benomyl, captan, and chlorothalonil, commonly used in current agricultural systems, were chosen to assess their impact on soil ecological processes and to establish harmonized approaches to evaluating their effects. Three experimental stages, laboratory batch incubations, a microcosm technique and a field study were included in evaluating the effects of the three fungicides on soil ecological processes by measuring: (1) soil microbial activity, including substrate-induced soil respiration (SIR) and soil enzymatic activities, (2) nitrogen dynamics, including NH$_4$-N, NO$_3$-N, dissolved organic nitrogen (DON) and microbial biomass nitrogen concentrations (BION), (4) nitrogen transformations including net N mineralization and nitrification, (5) rates of litter decomposition, (6) leaching of nutrients and (7) plant growth and yield. The effects of the fungicides on soil microbial activity, nitrogen dynamics and plant growth depended on the nature and concentrations of the fungicides, the quality of organic amendments, soil types and environmental conditions. Patterns of response of soil microbial activity and nutrient cycling processes to the chemicals were specific to each of the fungicides. Benomyl enhanced soil microbial activity and transformations of NH$_4$-N and DON, that resulted in
greater plant biomass and shoot heights. Captan suppressed N mineralization, apparently by reducing microbial activity, as indicated by short-term reductions in SIR and soil dehydrogenase activity. Significant accumulations of soil NH$_4$-N, over 90 days in the captan-treated soils, probably resulted from lower rates of nitrification. Soil microbial activity/biomass and in situ NO$_3$-N concentrations were depressed by the chlorothalonil treatment that resulted in retarded plant growth. Among the three fungicides, captan appeared to have more pronounced overall impact on soil microbial processes than either benomyl or chlorothalonil. The impact of benomyl at typical field application rates on soil microbial processes and the impact on soil environments was much less than that of the other fungicides. Taken together, the techniques and data presented in this study provided not only a much better understanding of the effects of soil applied fungicides and ecological processes, but also an idea how the interaction between the processes influence nutrient availability and plant growth in agricultural systems.
Dedicated to Ming-Ching, Shinru and my parents
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CHAPTER 1

LITERATURE REVIEW

Importance of the research
Pesticides have been sprayed on soils to control pests in agriculture. Herbicides, insecticides and fungicides are the main types of pesticides being used. The pesticide market grew very rapidly between 1960 and 1980 and the market is still growing. In 1991, 20.4% of pesticides used in the world were fungicides. Total amount of money spent on use of fungicides increases from 340 millions in 1960 to 5426 million of US dollars in 1994 (Tarradellas and Bitton 1997). Fungicides are used commercially in most in Western Europe which consists of 47% of the whole market share in the world. Moreover, about half of the fungicides are used on fruits and vegetables including vine (Lyr 1995). Some fungicides, like other kinds of pesticides are spread directly onto the soil and they act by inhibition of different biochemical functions including electron transport, synthesis and germination. Furthermore, fungicides, have a potential for killing or affecting the function of a diverse range of soil organisms that contribute to the biological processes and maintain soil structure and fertility (e.g. Edwards, 1988; Edwards and Bater, 1990; Edwards et al., 1996; Vyas, 1988). It is important to avoid contamination of soils by chemicals that have deleterious effects on soil processes and thereby affect soil health, for maintaining overall soil fertility. The fertility of soil, i.e. the capacity to produce more or less plentiful crops, depends not only on its physical constitution and its stock of nutrients, but also on the intensity of the biological processes that take place within it. The
increasing usage of pesticides, although intended to protect the crops, may alter biological equilibrium of the environment, by direct or indirect action, after a short, average, or long period of time, depending on whether the product acts quickly or persists longer in its initial state or in its metabolic forms (Simon-Sylvestre and Fournier, 1979).

The prediction of side effects of the application of pesticides on non-target microorganisms has already been a point of discussion for more than two decades and is still considered a difficult problem (Domsch 1991). There is an increasing awareness of possible effects of anthropogenic activities on the cultivated soil through unintended deposition of hazardous substances, thus, it is important the activities of soil microorganisms are not disturbed by anthropogenic activities (Torstensson 1997). The literature on the effects of chemicals on soil organisms and soil processes is extremely diverse, ranging from reports of the effects of chemicals on individual species of organisms, to those on overall populations of organisms and on individual biological systems using a great diversity of methods in both field and laboratory (Edwards and Bater, 1990). In addition, very little research has been done to examine the longer-term impact of fungicides on soil microbial activity and ecological processes (Tu, 1993) and little information is available to assess the effects of fungicides in microcosm study or under field conditions (Takagi et al., 1991). Moreover, many experiments have been done in vitro, involving unrealistic doses of chemicals, methods of application, and modes of exposure, so it is impossible to compare the results of experiments by different workers using the same chemicals and to reach a valid conclusion. Registration authorities have an urgent need for standardized or harmonized methodologies that will assess adequately the potential hazard of any pesticides in soil ecosystems. Hence, there is a need for integrated, innovative testing methodologies involving key processes in soils (Edwards, 1988).

The main purpose of my research proposal was to use key indicator processes and available methodologies that could be used to assess the effects of fungicides on particular soil processes and to standardize the methodologies for evaluating potential hazard and impact of pesticides as well as other soil pollutants to soil systems.
Importance of the microcosm approach for assessing ecological impacts of soil contaminants

Microcosms are defined as "a controlled, reproducible laboratory system which attempts to simulate the situation (i.e. processes and interaction of components) in a portion of the real world" (Gillett and Witt 1979). Among the literature, a soil microcosm is defined as "a replicable, experimental unit containing soil, in which the response of more than one biotic species is measured and where at least one of the biotic species is larger than microbial" (Sheppard 1997). Microcosm tests involve more species and likely involve more levels of biological organization and more endpoints than single-species tests and they may allow longer exposure and may be more realistic.

The need for microcosm studies originated from two important considerations: the fear of oversimplification and the problems associated with using xenobiotics or pollutants in field studies (Pritchard and Bourquin, 1984). Standardized tests for assessing the potential impacts of a toxicant to an ecosystem often involve single species or pure culture, and isolations of microorganisms from complex nature. The justification is that the interactions among populations may be more sensitive to toxicants and that community studies may ultimately lead to a more quantitative estimation of ecosystem effects. The microcosm is perceived as a potential laboratory tool that could help reduce this "fear of simplicity" by permitting fate and effects studies in much more ecologically complex laboratory systems. In addition, the microcosm gives researchers the opportunity to alter environmental conditions to simulate a large array of possible perturbations to the system. These options are usually not available in field studies. Moreover, the relatively small size of the microcosm permits replication, simplified dosing mechanics, control over inputs and outputs from the system, and adequate mixing, all factors are difficult to control in the field (Edwards et al., 1994; Pritchard and Bourquin, 1984).

Standardized methods are needed for accurately evaluating the impact of pesticides on soil microorganisms, which play an essential role in soil biological processes including nitrogen transformation, organic matter decomposition and nutrient availability, are necessary. In recent years, interest has developed in the use of microcosm technique for
ecotoxicological assays (Blair et al., 1989; Bogomolov et al., 1996; Draggan, 1977; Edwards et al., 1994; Gillett, 1989; Jackson et al., 1977; O’Neill et al., 1977; Parmelee et al., 1993; Sheppard 1997; Van Voris et al., 1985). Such microcosms have consisted of soil units containing multiple biotic species, and have ranged in size from a few grams of soil to as large as a meter in diameter. The microcosm approaches can assess the impact of contaminants at different levels of biological organization and produce data that is more relevant to field situations. Moreover, they have advantages of being relatively simple, inexpensive and reproducible.

Three types of microcosm devices can be distinguished (Heath, 1980; Ausmus et al., 1980): a closed type without free contact to air and moisture; a semi-open type with exposure only to air or moisture; and an open-type microcosm with free interaction with air and moisture. The microcosm unit used in this research can be identified as open type systems. Microcosms differed from the field situation concerning the influence of temperature and moisture dynamics, the influence of root presence, and the composition of the soil flora and fauna (Teuben and Verhoef, 1992). Many “ecosystem-level” behaviors are microbiologically based processes that reflect the integration of many internal processes and components and their interactions within ecosystems (Schindler et al., 1980). These behaviors may include community respiration, nutrient cycling, oxidation-reduction gradients, organic matter transformation, and primary and secondary production. All these properties cannot be inferred or predicted from measurements of isolated components such as single species or mixed cultures in the laboratory (Weiss, 1971).

Several measurements have been considered as good monitoring points to detect pollutant-induced changes in ecosystem behavior (Edwards et al., 1996; O’Neill et al., 1977; Pritchard and Bourquin, 1984; Van Voris et al., 1980). Leaching rates of nutrients from soil (O’Neill et al., 1977), CO$_2$ efflux from the respiration of soil microorganisms (Van Voris et al., 1980), effects on the nitrogen cycle and redox potential (Eh) (Pritchard and Bourquin 1984). Disturbing the redox gradient could lead to significant changes in metabolic rates of carbon turnover and mineralization by certain microbial communities. However, it is difficult to assess the influence of pollutants on soil ecosystems without
considering their potential interactive effects because soil organisms, including soil microorganisms and nutrient cycling processes, are so intimately linked. Single-process tests have been used to date in soil ecotoxicological research and environmental risk assessment could not provide full picture of data which can have accurate prediction of potential environmental hazards of pollutants in field conditions (Edwards et al., 1996). An integrated soil microcosm of the type we described in this paper could probably, provide the best information for overall predictions of potentially serious impacts of pollutants on soil ecosystems.

**Basic characteristics of tested fungicides, benomyl, captan and chlorothalonil**

**Benomyl** (Benlate®, DuPont 1991®, etc.), methyl-1-(buty carbamoyl)-2-benzimidazole carbamate (Fig. 1.1), is the first systemic fungicide, introduced in 1967 (Howard 1991) and controls a wide range of diseases of fruits, nuts, vegetables, field crops, turf and ornamentals (Mortvedt et al., 1989). Systematic fungicides such as benomyl have been used extensively through foliar and fruit application for the control of several plant pathogens in vegetable and fruit crops. However, they reach soil and persist there for a long time or they are exudated through the roots after foliar application (Vyas 1988). Benomyl has low solubility, and practically is insoluble in water or oil (Mortvedt et al., 1989). Log K_w (octanol/water partition coefficient) ranges from 2.12 to 3.11 (Howard 1991). Benomyl released on or into soil will not move downward or leach extensively because movement of benomyl through the soil profile does not exceed 20-35 cm with an annual rainfall of 150 cm (Haque and Freed 1974). Volatilization of benomyl from soil may be significant (Howard 1991), which ranges from 3.5 to 6.5 kg ha\(^{-1}\) y\(^{-1}\) or more (Haque and Freed 1974). Due to quick hydrolysis of benomyl to methylbenzimidazole-2-ylcarbamate (MBC) which is also a fungicidal compound (Menge 1982), half-life of benomyl ranges from 15 days in unsterilized soil to 6-12 months on bare soil (Howard 1991). Mechanism of antifungal action of benomyl is to affect biosynthesis of DNA in mitosis (Spencer 1977).

**Captan** (Merpan, Orthocide 406®, Vancide 89®, SR-406®, etc.), N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide (Fig. 1.1), is a protectant-eradicant
fungicide that belongs to a group of trichloromethylthiodicarboximides containing -
NSCCh$_3$ group (Spencer 1977). Captain controls (1) scab, black rot, botrytis, sooty blotch,
fly speck, summer rots on apples, (2) brown rot and leaf sport on stone fruits and
almonds, (3) dead arm, downy mildew, black rot on grapes, (4) wide variety of fungus
diseases on small fruits, berries, ornamentals, vegetables. Captain can also be used as seed
treatments by slurry, dry treatment, and plant-box application. Captain is compatible with
most commonly used pesticides and fungicides but can't be used with strong alkalies and
oil sprays (Mortvedt et al., 1989). Mechanism of antifungal action of captain is primarily
due to the R-SCCl$_3$ groups which are fungitoxic (Sinha et al., 1988). Solubility of captain
is 0.5 mg l$^{-1}$ and log K$_{ow}$ of captain ranges from 1.5 to 2.54 (Briggs, 1981; Howard 1991;
Leo et al., 1971; Rao and Davidson 1980; Sunito et al., 1988). Captain released to soil will
be moderately mobile, but it may not leach into ground water as it generally degrades fast
in soil. Captain persists longer in soil when localized in high concentrations than when
uniformly distributed through soil (Griffith and Matthew 1969). Depending on soil pH,
type and moisture content, the half-life of captain in different soils varies (Howard 1991).
The half-life of captain in moist soil ranges from 1-12 days (Howard 1991); however,
some showed that captain applied at 250 mg kg$^{-1}$ had half-life of 70 days (Brown 1978).
Volatilization of captain may be slow because of evaporation rate from a loam soil was 0.2
to 3.0 kg ha$^{-1}$ y$^{-1}$ under annual rainfall of 150 cm (Haque and Freed 1974).

Chlorothalonil (Bravo, Daconil® 2787, Clortocaf Ramato, Exotherm Termil etc),
tetrachloroisophthalonitrile (Fig. 1.1), compared to benomyl and captain, is a relatively
new fungicide which controls broad ranges of diseases of beans, carrot, celery, cole crops,
conifers, corn grown for seed, sweet corn, cranberry, cucumbers, dry edible beans, garlic,
leek, melons (cantaloupe, muskmelon, honeydew, watermelon), mint, onion, papaya,
passion fruit, peanut, potato, pumpkin, shallot, snap beans, soybeans, squash, stone fruits,
tomato, ornamentals and grass grown for seed (Mortvedt et al., 1989; Spencer 1977) and
as a wood preservative (Laks et al., 1992). Solubility of chlorothalonil is low (0.6 mg kg$^{-1}$)
and only slightly soluble in xylene and acetone (Mortvedt et al., 1989). Mechanism of
antifungal action of chlorothalonil is attributed to thiol inactivation (Vincent and Sisler 1968).

\[
\text{CONH-}-(\text{CH}_2)_3\text{CH}_3
\]

\[
\text{N} \quad \text{N} \quad \text{COOCH}_3
\]

I. Benomyl

\[
\text{O} \\
\text{N} \\ \\
\text{SCCl}_3
\]

II. Captan

\[
\text{Cl} \\
\text{Cl} \\
\text{Cl} \\
\text{CN}
\]

III. Chlorothalonil

Figure 1.1: Chemical structure of benomyl, captan and chlorothalonil.

**Effects of benomyl, captan and chlorothalonil on soil microbial activity**

**Soil respiration**

Microbial activity is generally considered to be an index of soil fertility by measuring actual activity in the field or potential activity in the laboratory (Parkinson and Coleman 1991). There has been an increase in the frequency of using CO\(_2\) evolution measurements in assessing soil microbial activity. Soil respiration has been considered as an indicator of overall soil activity, which is measured by either O\(_2\) consumption or CO\(_2\) evolution (Vyas 1988). Studies showed that with soil fungicides, soil respiration usually is inhibited for a short time after application, but surviving microflora recover soon and the respiration rate rises to high levels, even exceeding those of untreated soil (Domsch 1964). Effects of our testing fungicides on soil respiration are listed in table 1.1, 1.2 and 1.3 for benomyl, captan
and chlorothalonil, respectively. Most of the literature regarding benomyl showed that benomyl increased CO$_2$ evolution rates due to serving as a substrate and utilized by soil microorganisms (Table 1.1). Studies concerning influences of captan on soil respiration are controversial (Table 1.2). For example, captan at 20 or 200 mg kg$^{-1}$ showed a periodically limited CO$_2$ overproduction which was followed by a significant inhibition. On the other hand, soils treated with 2 mg kg$^{-1}$ captan showed stimulatory effects on CO$_2$ evolution (Zelles et al., 1985). The literature regarding the effects of chlorothalonil on soil respiration was too sparse to make any conclusions. Furthermore, known results are also controversial (Table 1.3).

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Measurements</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2 and 22.4 kg a.i. ha$^{-1}$</td>
<td>CO$_2$ production</td>
<td>increase</td>
<td>Peeples 1974</td>
</tr>
<tr>
<td>50 and 200 mg kg$^{-1}$</td>
<td>CO$_2$ evolution</td>
<td>increase</td>
<td>Van Faassen 1974</td>
</tr>
<tr>
<td>3-30 kg ha$^{-1}$</td>
<td>CO$_2$ evolution</td>
<td>increase</td>
<td>Hofer et al., 1971</td>
</tr>
<tr>
<td>N/A</td>
<td>CO$_2$ evolution</td>
<td>increase</td>
<td>Sinha et al., 1988</td>
</tr>
<tr>
<td>N/A</td>
<td>respiration</td>
<td>decrease</td>
<td>Weeks and Hedrick 1975 in Simon-Sylvestre and Fournier 1979</td>
</tr>
</tbody>
</table>

Table 1.1: Effects of benomyl on soil respiration
### Concentrations Measurements Effects Reference

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Measurements</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>recommended rates</td>
<td>soil respiration</td>
<td>decrease then increase</td>
<td>Domsch 1964</td>
</tr>
<tr>
<td>62.5, 125, 250 mg kg⁻¹</td>
<td>CO₂ production</td>
<td>decrease</td>
<td>Agnihotri 1971</td>
</tr>
<tr>
<td>2 mg kg⁻¹</td>
<td>CO₂ evolution</td>
<td>increase</td>
<td>Zelles et al., 1985</td>
</tr>
<tr>
<td>20 and 200 mg kg⁻¹</td>
<td></td>
<td>decrease/inhibition</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>respiration</td>
<td>decrease</td>
<td>Domsch 1970</td>
</tr>
<tr>
<td>500 μg g⁻¹</td>
<td>soil respiration</td>
<td>increase</td>
<td>Domsch 1964, 1965</td>
</tr>
</tbody>
</table>

Table 1.2: Effects of captan on soil respiration

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Measurements</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μg g⁻¹ (99.7 % purity)</td>
<td>O₂ consumption</td>
<td>increase</td>
<td>Tu 1993</td>
</tr>
<tr>
<td>0.4 kg acre⁻¹</td>
<td>CO₂ evolution</td>
<td>decrease</td>
<td>Takai et al., 1987</td>
</tr>
</tbody>
</table>

Table 1.3: Effects of chlorothalonil on soil respiration

**Enzyme activities**

In comparison with soil respiration, using soil enzymes in assessing soil microbial activity has been much less frequent. However, these measurements provide valuable data on the biochemical properties of soils under different climatic or tillage regimes (Parkinson and Coleman 1991). Enzymes are protein catalysts that promote chemical reactions without undergoing permanent alteration (Dick and Tabatabai 1992). Soil enzymes are mostly extracellular enzymes, to some extent stabilized against unfavorable conditions and partially independent of changes occurring in the living organisms (Cervelli et al., 1978).
Main sources of soil enzymes are from microorganisms and plants. The production of extracellular enzymes allows microorganisms to initiate the degradation of higher molecular weight organic macromolecules and the oligomers produced are used by microbes or plants (Rossel et al., 1997). Majority of microbial enzymes are produced by fungi in soil (Paul and Clark 1988). Major groups of soil enzymes include oxidoreductases (e.g. dehydrogenase, catalases, peroxidases, phenol oxidases and glucose oxidases), hydrolase (e.g. phosphatase, ureases, cellulases, proteases, xylanases, nucleases and β-galactosidase), transferases, isomerases, invertases and lyases (Dick and Tabatabai 1992; Skujins 1976). Some researchers claimed that the most valuable single use of soil enzymes is to assess the effects of pollution on the soil health (Dick and Tabatabai 1992). The most frequently used enzymes are urease, protease, catalase, β-galactosidase, arylsulfatase, saccharase and phosphodiesterase (Rossel et al., 1997). An outline of effects of fungicides (pesticides) on soil enzymes is presented in figure 1.2 (Cervelli et al., 1978). Many studies involved using pesticides at the recommended field application rates and found that inhibitory effects of pesticides on soil enzymes were temporary and enzyme activities return to levels similar to those in untreated soils. (Dick and Tabatabai 1992). Fungicides, like other pesticides may affect soil microorganisms by modifying biosynthetic mechanisms and protein biosynthesis, disorganizing physical structure of plasma membranes, altering transport or excretion of membranes, and influencing transport of plant growth regulators (Cervelli et al., 1978).

Effects of benomyl, captan and chlorothalonil on soil enzyme activities found in the literature are listed in Table 1.4, 1.5 and 1.6, respectively. Most studies measured two major enzymes, dehydrogenase and phosphatase to evaluate effects of pesticides on soil microbial activity. Contrary to other soil enzymes, dehydrogenases are intracellular and their degradation in soils appears to be very rapid following cell death, thus they do not accumulate in soils (Skujins 1976; Rossel et al., 1997). Numerous reports regarding correlation between dehydrogenase and biomass, or between the impact of chemicals on dehydrogenase and on the biomass of soil microflora, confirm that dehydrogenase is an index of biomass of the physiologically active biomass of soil microflora (Rossel et al.,
Phosphatases are not related to microbial biomass and the respiratory activity and are only sometimes related to the available P content of soil (Skujins 1976). Among the three tested fungicides, benomyl had less influences on soil enzyme activities compared to captan and chlorothalonil. Captan, on the other hand, increased dehydrogenase activity and reduced activities of phosphatase and enzymes related to nitrogen cycling (e.g. L-asparaginase and L-glutaminase) in soils (Table 1.5). Captan did not have specific effects on urease, invertase, amylase and aminase. Chlorothalonil had significant impacts on enzymes related to carbon cycling. Activities of cellulase, carboxyl esterase, invertase and amylase were depressed by chlorothalonil in various soils (Katayama and Kuwatsuka 1991; Nakamura et al., 1990; Tu 1993). However, chlorothalonil stimulated activities of dehydrogenase and phosphatase in the soil (Table 1.6).

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Enzymes</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-42 kg a.i. ha⁻¹</td>
<td>dehydrogenase</td>
<td>increase</td>
<td>Li and Nelson 1985</td>
</tr>
<tr>
<td>20 and 200 mg kg⁻¹</td>
<td>dehydrogenase, urease</td>
<td>no effect</td>
<td>Van Faassen 1974</td>
</tr>
<tr>
<td>3.0-30.0 kg ha⁻¹</td>
<td>dehydrogenase, amylase, catalase</td>
<td>no effect</td>
<td>Hofer et al., 1971</td>
</tr>
<tr>
<td>100-10000 µg g⁻¹</td>
<td>dehydrogenase, xylanase, urease</td>
<td>decrease</td>
<td>Gowda 1973</td>
</tr>
<tr>
<td>recommended field rate</td>
<td>dehydrogenase, xylanase, urease</td>
<td>no effect</td>
<td>Mitterer et al., 1981</td>
</tr>
<tr>
<td>N/A</td>
<td>dehydrogenase</td>
<td>no effect</td>
<td>Chendrayan and Sethunathan 1980</td>
</tr>
</tbody>
</table>

Table 1.4. Effects of benomyl on soil enzyme activities.
Fig. 1.2: An outline of effects of fungicides on soil microorganisms and activities of soil enzymes.
<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Enzymes</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 and 10 µg g⁻¹</td>
<td>urease</td>
<td>increase</td>
<td>Tu 1981 b</td>
</tr>
<tr>
<td>14-42 kg a.i. ha⁻¹</td>
<td>dehydrogenase</td>
<td>increase</td>
<td>Li and Nelson 1985</td>
</tr>
<tr>
<td>10 mg kg⁻¹</td>
<td>L-asparaginase</td>
<td>decrease</td>
<td>Frankenberger and Tabatabai 1991 a</td>
</tr>
<tr>
<td>recommended field rate</td>
<td>urease</td>
<td>no effect</td>
<td>Mitterer et al., 1981</td>
</tr>
<tr>
<td>5 and 10 µg g⁻¹</td>
<td>dehydrogenase</td>
<td>increase</td>
<td>Tu 1981 a, b</td>
</tr>
<tr>
<td>5 and 10 µg g⁻¹</td>
<td>phosphatase</td>
<td>no effect or decrease</td>
<td>Tu 1981 a, b</td>
</tr>
<tr>
<td>5 and 10 µg g⁻¹</td>
<td>invertase, amylase</td>
<td>no effect</td>
<td>Tu 1982</td>
</tr>
<tr>
<td></td>
<td>aminase</td>
<td>no effect</td>
<td>Frankenberger and Tabatabai 1981</td>
</tr>
<tr>
<td></td>
<td>L-glutaminase</td>
<td>decrease</td>
<td>Frankenberger and Tabatabai 1991 b</td>
</tr>
</tbody>
</table>

Table 1.5: Effects of captan on soil enzyme activities.
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Enzymes</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mg kg⁻¹</td>
<td>acid phosphatase</td>
<td>increase</td>
<td>El Hissy et al., 1995¹</td>
</tr>
<tr>
<td>4 kg 10 a⁻¹</td>
<td>dehydrogenase</td>
<td>increase</td>
<td>Takai et al., 1987</td>
</tr>
<tr>
<td>10 μg a.i. g⁻¹</td>
<td>invertase, amylase, phosphatase, and dehydrogenase</td>
<td>decrease</td>
<td>Tu 1993</td>
</tr>
<tr>
<td>recommended field rate</td>
<td>phosphatase and amidase</td>
<td>no effect</td>
<td>Nakamura et al., 1990</td>
</tr>
<tr>
<td></td>
<td>aryl esterase</td>
<td>slight decrease</td>
<td>Nakamura et al., 1990</td>
</tr>
<tr>
<td></td>
<td>carboxyl esterase</td>
<td>decrease</td>
<td>Nakamura et al., 1990</td>
</tr>
<tr>
<td></td>
<td>cellulase</td>
<td>decrease</td>
<td>Katayama and Kuwatsuka 1991</td>
</tr>
</tbody>
</table>

1. Results were obtained in an aquatic system.

Table 1.6: Effects of chlorothalonil on soil enzyme activities.

**Effects of benomyl, captan and chlorothalonil on soil microbial populations**

Fungicides generally do not affect soil chemoheterotrophic and chemoaerotrophic bacteria and actinomycetes, but can inhibit Rhizobia, photosynthetic bacteria, saprophytic fungi, mycorrhizae, algae and protozoa (Hicks et al., 1990). Effects of benomyl, captan and chlorothalonil on soil microbial populations, as well as microbial biomass, are listed in table 1.7, 1.8 and 1.9, respectively. Benomyl increased bacterial and fungal populations in soils although some studies showed benomyl reduced fungal populations. The most affected fungal groups are probably the Deuteromycetes (Edgington et al., 1971) and the most affected bacterial groups are the nitrifiers (Van Faassen 1974). In field studies,
Benomyl has a tendency to increase both bacterial and fungal numbers as well as actinomycetes. Good correlation between elevated microbial activity and increasing microbial numbers may indicate that benomyl is utilized by soil microorganisms as a substrate source.

Reports about effects of captan on soil microorganisms are enormous and show that captan tends to suppress populations of fungi and actinomycetes but to enhance bacterial numbers. However, controversial results were obtained from the literature due to different testing conditions, soil properties and concentrations of captan. Captan at higher concentrations apparently has more deleterious effects on soil microorganisms especially soil saprophytic fungi and nitrifying bacteria (Ingham 1985). Similar to captan, chlorothalonil reduces populations of fungi and actinomycetes but increases total bacterial numbers in the soils (Takei et al., 1987; Tu 1993).

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Measurements</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.2 kg ha(^{-1}) (foliage or drench applied)</td>
<td>total bacteria, actinomycetes and fungi</td>
<td>no effect</td>
<td>Smiley and Craven 1979</td>
</tr>
<tr>
<td>50 and 100 µg g(^{-1})</td>
<td>bacteria</td>
<td>increase</td>
<td>Weeks and Hedrick 1975</td>
</tr>
<tr>
<td>4, 20 kg ha(^{-1})</td>
<td>bacteria and fungi</td>
<td>increase</td>
<td>Wainwright and Pugh 1974</td>
</tr>
<tr>
<td>50 and 200 mg kg(^{-1})</td>
<td>bacteria and actinomycetes</td>
<td>increase</td>
<td>Van Faassen 1974</td>
</tr>
<tr>
<td>N/A</td>
<td>bacteria and actinomycetes</td>
<td>increase</td>
<td>Simon-Sylvestre and Fournier 1979</td>
</tr>
<tr>
<td>0.1-10 kg ha(^{-1})</td>
<td>fungi and actinomycetes</td>
<td>increase</td>
<td>Raynal and Ferrari 1973 in Anderson 1978</td>
</tr>
</tbody>
</table>

Table 1.7: Effects of benomyl on soil microbial populations.
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Response</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 μg g⁻¹</td>
<td>bacteria and actinomycetes</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brown 1978</td>
</tr>
<tr>
<td>0.01-100 mg l⁻¹ in agar</td>
<td>fungi (especially Deuteromycetes)</td>
<td>decrease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Edgington et al., 1971</td>
</tr>
<tr>
<td>0-200 mg kg⁻¹ (50% a.i.)</td>
<td>nitrifera</td>
<td>decrease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Van Faassen 1974</td>
</tr>
<tr>
<td>3-30 kg ha⁻¹</td>
<td>numbers of fungi, bacteria and actinomycetes</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hofer et al., 1971</td>
</tr>
<tr>
<td>2.2 kg a.i. ha⁻¹</td>
<td>fungi</td>
<td>increase then decrease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peeples 1974</td>
</tr>
<tr>
<td>2.2 and 89.6 kg a.i. ha⁻¹</td>
<td>bacteria</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peeples 1974</td>
</tr>
<tr>
<td>89.6 kg a.i. ha⁻¹</td>
<td>fungi</td>
<td>decrease then increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peeples 1974</td>
</tr>
<tr>
<td>2.2 and 22.4 kg a.i. ha⁻¹</td>
<td>bacteira</td>
<td>increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peeples 1974</td>
</tr>
<tr>
<td>2.2 and 22.4 kg a.i. ha⁻¹</td>
<td>fungi</td>
<td>increase then decrease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peeples 1974</td>
</tr>
<tr>
<td>Concentrations</td>
<td>Measurements</td>
<td>Effects</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>62.5, 125 and 250 mg kg(^{-1})</td>
<td>fungi and actinomycetes</td>
<td>decrease</td>
</tr>
<tr>
<td>62.5, 125 and 250 mg kg(^{-1})</td>
<td>aerobic spore forming bacteria</td>
<td>increase</td>
</tr>
<tr>
<td>25 (\mu g \text{ g}^{-1})</td>
<td>total bacteria</td>
<td>no effect</td>
</tr>
<tr>
<td>25 (\mu g \text{ g}^{-1})</td>
<td>total fungi (hyphal length) and total bacteria</td>
<td>increase then</td>
</tr>
<tr>
<td>175 kg ha(^{-1})</td>
<td>fungal hyphae and fungal CFU(^1)</td>
<td>reduce</td>
</tr>
<tr>
<td>17.5 g m(^2)</td>
<td>total fungal hyphal length</td>
<td>reduce</td>
</tr>
<tr>
<td>25 (\mu g \text{ g}^{-1})</td>
<td>active fungi (FDA stained hyphal length)</td>
<td>decrease</td>
</tr>
<tr>
<td>25 (\mu g \text{ g}^{-1})</td>
<td>total fungi (JM) and viable bacterial (T and N)(^3)</td>
<td>no effect</td>
</tr>
<tr>
<td>25 (\mu g \text{ g}^{-1})</td>
<td>viable fungi (CFU)</td>
<td>increase</td>
</tr>
<tr>
<td>2-10 kg ha(^{-1})</td>
<td>total platable fungal populations, nitrifying bacteria and aerobic N(_2)-fixing bacteria</td>
<td>decrease</td>
</tr>
<tr>
<td>9 kg ha(^{-1})</td>
<td>fungal propagules</td>
<td>decrease</td>
</tr>
</tbody>
</table>

Table 1.8: Effects of captan on soil microbial populations.
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Effect</th>
<th>Authors and Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 μg g⁻¹</td>
<td>total or viable bacteria and total fungi</td>
<td>no effect</td>
</tr>
<tr>
<td>9 kg ha⁻¹</td>
<td>bacteria, fungi</td>
<td>increase</td>
</tr>
<tr>
<td>N/A</td>
<td>bacteria, actinomycetes</td>
<td>increase</td>
</tr>
<tr>
<td>5.26 kg ha⁻¹</td>
<td>fungal numbers</td>
<td>decrease</td>
</tr>
<tr>
<td>5.26 kg ha⁻¹</td>
<td>cellulolytic fungal species, bacterial numbers</td>
<td>increase</td>
</tr>
<tr>
<td>5-100 μg g⁻¹</td>
<td>actinomycetes</td>
<td>increase or decrease</td>
</tr>
<tr>
<td>10 mg kg⁻¹</td>
<td>bacterial numbers</td>
<td>increase</td>
</tr>
<tr>
<td>10 mg kg⁻¹</td>
<td>fungal numbers</td>
<td>increase</td>
</tr>
<tr>
<td>2 and 20 mg kg⁻¹</td>
<td>FDA and ATP</td>
<td>increase</td>
</tr>
<tr>
<td>200 mg kg⁻¹</td>
<td>FDA and ATP</td>
<td>decrease</td>
</tr>
<tr>
<td>1, 10 and 100 μg g⁻¹</td>
<td>actinomycetes</td>
<td>no effect</td>
</tr>
<tr>
<td>1, 10 and 100 μg g⁻¹</td>
<td>bacterial and fungal numbers and nitrifiers</td>
<td>decrease</td>
</tr>
<tr>
<td>N/A</td>
<td>bacteria, actinomycetes</td>
<td>increase</td>
</tr>
<tr>
<td>3 and 10 μg g⁻¹</td>
<td>total bacteria</td>
<td>increase</td>
</tr>
</tbody>
</table>

[to be continued]
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Organism</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 and 10 μg g⁻¹</td>
<td>total fungi</td>
<td>decrease then increase</td>
<td>Tu 1981b</td>
</tr>
<tr>
<td>0.5-10 kg ha⁻¹</td>
<td>nitrifiers</td>
<td>increase</td>
<td>Nesheim and Linn 1968</td>
</tr>
<tr>
<td>25 kg ha⁻¹</td>
<td>nitrifiers</td>
<td>decrease</td>
<td>Nesheim and Linn 1968</td>
</tr>
<tr>
<td>1-1000 mg l⁻¹</td>
<td>bacteria</td>
<td>increase</td>
<td>Chinn 1973</td>
</tr>
<tr>
<td>75% a.i.</td>
<td>bacteria</td>
<td>increase</td>
<td>Naumann 1971 in Anderson 1978</td>
</tr>
</tbody>
</table>

1. CFU stands for colony forming unit.
2. FDA stands for fluorescein diacetate-stained hyphal length; FITC stands for fluorescein isothiocyanate staining technique.
3. JM stands for total hyphal length; T stands for bacterial plate count (tryptone); and N stands for bacterial plate count (nutrient agar).
4. Effects are concentration dependent.
5. ATP stands for Adenosine triphosphate.
6. Numbers are recovered after initial decrease.
7. Bacteria include *E. coli*, *Bacillus subtilis*, *Sarcina*, *Streptomyces*, *Cochliobolus*, *Ulocladium*. 
Table 1.9: Effects of chlorothalonil on soil microbial populations.

Effects of benomyl, captan and chlorothalonil on nitrogen cycling—primarily on nitrogen transformations

Availability of nutrients for plants in soils depends very crucially on soil microorganisms and their activities. The extent of microbial activity relies upon the balance between microbial mobilization and immobilization (Vyas 1988). The extensive use of fungicides has created a series of concerns regarding soil health and crop growth. Any fungicide that has an effect on soil microorganisms involved in decomposition of organic matter, mineralization and/or nitrification is likely to have corresponding effects on soil fertility and plant nutrition. The effects of benomyl, captan and chlorothalonil on soil nitrogen transformations are listed in table 1.10 and 1.11, respectively. The main concern of the research is on the availability of NH$_4$-N and NO$_3$-N, mineralization and nitrification. Benomyl applied at 4-20 kg ha$^{-1}$ increased NH$_4$-N but decrease NO$_3$-N concentrations in the soil (Wainwright and Pugh 1974). However, benomyl did not affect soil nitrification in field conditions (Mazur and Hughes 1975). The differences in the effects of benomyl on the nitrification and mineralization of nitrogen in the laboratory as compared to field application were ascribed to more rapid rates of degradation under field
conditions as contrasted with the high rate of a single application under laboratory conditions (Muzur and Hughes 1975; Vyas 1988). Ramakrishna et al (1979) used *Nitrosomonas* sp. and *Nitrobacter agilis* and found that benomyl increased nitrification by *Nitrobacter agilis* but depress nitrification by *Nitrosomonas* sp.

The effects of captan on soil nitrogen transformations were very consistent throughout the literature no matter what concentrations were applied to soils (Table 1.11). Applications of captan decreased amount of NO$_3$-N in the soil and suppressed soil nitrification (Agnihotri 1971; Wainwright and Pugh 1973, 1974; Mahmoud et al., 1972). On the other hand, captan increased release of NH$_4$-N in the soil and stimulated ammonification as well (Wainwright and Pugh 1973). Chlorothalonil is a relatively new fungicide and its effects on soil nitrogen transformation are very scarce. Tu (1993) found that chlorothalonil at 10 µg g$^{-1}$ reduced soil nitrification compared to the untreated controls.

**Effects of benomyl, captan and chlorothalonil on organic matter decomposition**

Organic matter decomposition is an emergent process of microbial communities (Sinsabaugh 1994). Each of the major insoluble components of plant litter require complex enzyme systems for complete degradation. Rates of decomposition principally should be correlated with enzymes activities responsible for degradation (Sinsabaugh 194). The effects of fungicides on the decomposition of crop residues have been mainly investigated under in vitro conditions. The process of decomposition depends on the composition of fungicide-resistant (tolerant) population, the kind of substrate to be decomposed, the persistence of the fungicide (Vyas 1988) and replacement of litter (Coleman et al., 1993). Surface litter is more available for decomposing than the buried litter (Beare et al., 1992, 1993; Coleman et al., 1993). Application of benomyl did not influence straw or organic matter decomposition in soils (Hofer et al., 1971; Torstenssen and Wessen 1984; Vyas 1988). However, benomyl at 100 µg g$^{-1}$ completely stopped the degradation of labeled cellulose in acid soils but had no effect in neutral soil (Hofer et al., 1971). In addition,
benomyl doses of 2 kg ha\(^{-1}\) in sandy soils inhibited early phase of straw decomposition (Torstenssen and Wessen 1984). The mass losses of buried pepperweed litter were decreased by treatments of benomyl and captan by 25% for litter and 15% for roots in semiarid dessert (Parker et al., 1984). One possible explanation is that benomyl acts primarily on deuteromycetes which are fast-growing fungi and nutrient-depending. On the contrary, the fungi which are least affected by benomyl are basidiomycetes, which are slow-growing fungi and not as nutrient-dependent (Torstenssen and Wessen 1984).

---

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Measurements</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.2 kg ha(^{-1})</td>
<td>NH(_4)-N</td>
<td>increase</td>
<td>Smiley and Craven 1979</td>
</tr>
<tr>
<td>2, 25, 75 and 150 mg kg(^{-1})</td>
<td>NH(_4)-N, NO(_2)- and NO(_3)-N</td>
<td>no effect</td>
<td>Mazur and Hughes 1975</td>
</tr>
<tr>
<td>4 and 20 kg ha(^{-1})</td>
<td>NH(_4)-N</td>
<td>increase</td>
<td>Wainwright and Pugh 1974</td>
</tr>
<tr>
<td>10, 25 and 100 mg kg(^{-1})</td>
<td>NO(_2)- and NO(_3)-N</td>
<td>increase</td>
<td>Van Faassen 1974</td>
</tr>
<tr>
<td>4, 20 kg ha(^{-1})</td>
<td>nitrification</td>
<td>decrease</td>
<td>Wainwright and Pugh 1974</td>
</tr>
<tr>
<td>3-30 kg ha(^{-1})</td>
<td>nitrification</td>
<td>decrease</td>
<td>Hofer et al., 1971</td>
</tr>
<tr>
<td>90 kg ha(^{-1})</td>
<td>nitrification</td>
<td>no effect¹</td>
<td>Mazur and Hughes 1975</td>
</tr>
<tr>
<td>10, 100 and 1000 mg kg(^{-1})</td>
<td>nitrification</td>
<td>decrease</td>
<td>Ramakrishna et al., 1979</td>
</tr>
<tr>
<td>(simulated oxidized surface)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 and 100 mg kg(^{-1})</td>
<td>nitrification by \textit{Nitrosomonas} sp.</td>
<td>decrease</td>
<td>Ramakrishna et al., 1979</td>
</tr>
<tr>
<td>10 and 100 mg kg(^{-1})</td>
<td>nitrification by \textit{Nitrobacter agilis}</td>
<td>increase</td>
<td>Ramakrishna et al., 1979</td>
</tr>
</tbody>
</table>

¹. nitrogen metabolism was enhanced by application of benomyl.

Table 1.10: Effects of benomyl on soil nitrogen transformations
Table 1.11: Effects of captan and chlorothalonil on soil nitrogen transformations

Broad-spectrum fungicides like captan can cause pronounced changes in soil microbial composition. Captan at the operational rates completely inhibited cellulose degradation for 6 weeks following treatment and had even more severe inhibitory influence on decomposition of chitin and cutin (Domsch 1970). Captan significantly slowed the decomposition of surface-buried sorghum but did not affect rates of buried sorghum residue decay (Beare et al., 1993). Moreover, captan applied at 17.5 g m\(^{-2}\), 47% a.i. to rye and grain sorghum residues in litter bags decreased fungal activity and total decomposition under field conditions (Beare et al., 1992; Coleman et al., 1993). There are
several reports regarding effects of chlorothalonil on cellulose decomposition in soils (Katayama and Kuwatsuka 1991; Sun et al., 1985; Suyama et al., 1993). Chlorothalonil completely inhibited cellulose decomposition at a concentration higher than 150 µg g⁻¹ soil under upland (Katayama and Kuwatsuka 1991; Sun et al., 1985), transitional flooded and flooded conditions (Katayama and Kuwatsuka 1991). Environmental conditions such as soil temperature dictate the effects of chlorothalonil on cellulose degradation. At two different temperature (13 and 25 °C), chlorothalonil caused retardation of cellulose decomposition depended on the concentrations of the fungicide. The higher temperature needs the higher concentration of fungicide to inhibit breakdown of cellulose. This retardative effect on cellulose decomposition seemed to be ascribed to reduction in the dominance of Rhizoctonia solani on the cellulose sheets (Suyama et al., 1993).

Effects of benomyl, captan and chlorothalonil on plant growth

Treatments of soils with fungicides may have a beneficial impact on plant growth because of correction of wide soil-borne phytostasis (Rawlinson and Calhoun 1970), increasing mobilization and availability of NH₄-N (Jenkinson et al., 1972), decreasing parasitic activity (Martin 1963) and mostly increasing release of plant nutrients, especially nitrogen, from organic and inorganic soil constituents (Smith 1963; Vyas 1988). Among the literature, benomyl increased crop yields and enhanced plant growth and biomass (Table 1.12), with the exception of onion treated with 0.4 mg ml⁻¹ of benomyl (De Bartoldi et al., 1977, 1978). Controversial results occurred in the literature about effects of captan on plant growth (Table 1.13). Captan enhanced growth of apple, Caribbean pine, Douglas fir, grain sorghum, longleaf pine, tomato and white clover, (Colinas et al., 1994; Fisher 1976; Hong 1976; Hu et al., 1995; Pawuk et al., 1980; Ross 1964; Somda et al., 1990, 1991). On the other hand, captan either did not cause significant increases in crop yields or plant growth of annual grasses, blue grama grass and watermelon (Ingham et al., 1986; Ingham and Coleman 1986; Liu et al., 1994), or decreased growth of Montetey pine and onion (De Baertoldi et al., 1977, 1978; Theodorou and Skinner 1976).
Researchers have claimed that the increases in growth, yield and other factors were mainly attributed to prolonged photosynthetic activity rather than to control of specific diseases (Vyas 1988). There were three possible mechanisms to explain possible phytotonic effects of fungicides on plants: (1) the fungicide per se directly affect physiology and growth, (2) control of weak pathogens may at critical times have large effects on yield and (3) leaf surface microflora may contribute to producing these effects (Dickinson 1981; Griffiths 1981; Melville and Jemmett 1971; Sijpesteijn 1977; Vyas 1988). Part of chemical structure of benomyl resembles kinetin, which gives benomyl cytokinin-like effects on plants such as increasing in protein synthesis and maintaining chlorophyll biosynthesis in detached leaves (Wang et al., 1961; Wang and Waygood 1959). However, the growth and yield responses may be results of complex interactions between fungicides and plants. The mechanisms involved are still unclear (Vyas 1988).
<table>
<thead>
<tr>
<th>concentrations</th>
<th>plant species</th>
<th>measurements</th>
<th>effects</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg pot⁻¹</td>
<td>tomato</td>
<td>root/shoot ratio</td>
<td>increase</td>
<td>Somda et al., 1991</td>
</tr>
<tr>
<td></td>
<td>'Better Boy'</td>
<td>total dry weight</td>
<td>increase by NS¹</td>
<td>1990</td>
</tr>
<tr>
<td>25 mg ka⁻¹</td>
<td>tomato</td>
<td>shoot dry weight</td>
<td>NS</td>
<td>Somda et al., 1990</td>
</tr>
<tr>
<td></td>
<td>'Better Boy'</td>
<td>root dry weight</td>
<td>increase</td>
<td>1990</td>
</tr>
<tr>
<td>0.4 mg ml⁻¹</td>
<td>onion</td>
<td>bulb diameters</td>
<td>decrease</td>
<td>De Bartoldi et al., 1977, 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dry weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25, 1.0, 2.0, 4.0 mg m⁻²</td>
<td>winter annual</td>
<td>total plant biomass</td>
<td>increase</td>
<td>Newsham et al., 1994</td>
</tr>
<tr>
<td></td>
<td>grass Vulpia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ciliata ssp.</td>
<td>No. of seeds/plant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ambigua</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>barley</td>
<td>yield</td>
<td>increase</td>
<td>Griffiths and Scott 1977</td>
</tr>
<tr>
<td>200-5000 mg kg⁻¹</td>
<td>wheat</td>
<td>No. of tillers, yield</td>
<td>increase</td>
<td>Peat and Shipp 1981</td>
</tr>
<tr>
<td>0.25% a.i. kg ha⁻¹</td>
<td>soybean</td>
<td>yield</td>
<td>increase</td>
<td>Horn et al., 1975</td>
</tr>
<tr>
<td></td>
<td>'Davis' &amp;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>'Lee-68'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15%</td>
<td>soybean</td>
<td>yield</td>
<td>increase</td>
<td>Julio et al., 1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of seeds and</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>pods per plant</td>
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</tr>
</tbody>
</table>

Table 1.12: Effects of benomyl on plant growth.
[Table 1.12 continued]

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>wheat</td>
<td>yield</td>
<td>increase</td>
<td>Cook 1977</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>barley</td>
<td>yield</td>
<td>increase</td>
<td>Hill and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lacey 1983</td>
<td></td>
</tr>
<tr>
<td>Field rates</td>
<td>winter wheat</td>
<td>yield</td>
<td>increase</td>
<td>Dickinson</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and Warploe</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1973</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>apple</td>
<td>yield</td>
<td>increase</td>
<td>Vyas 1988</td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td>longleaf pine</td>
<td>top weight</td>
<td>increase but NS</td>
<td>Pawuk et al.,</td>
<td></td>
</tr>
<tr>
<td>a.i./seedling in</td>
<td>root weight</td>
<td></td>
<td></td>
<td>1980</td>
<td></td>
</tr>
<tr>
<td>50 ml water</td>
<td>stem diameter</td>
<td>increase$^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td>longleaf pine</td>
<td>top weight</td>
<td>increase$^3$</td>
<td>Pawauk et</td>
<td></td>
</tr>
<tr>
<td>a.i./seedling in</td>
<td>root weight</td>
<td></td>
<td></td>
<td>al., 1980</td>
<td></td>
</tr>
<tr>
<td>50 ml water</td>
<td>stem diameter</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. NS means noy significant compared to controls.

2. Longleaf pine seedlings were infested with *Pisolithus tinctorius*.

3. Longleaf pine seedlings were not infested with *Pisolithus tinctorius* but were naturally infested by airborne fungi (mostly *Thelephora terrestris* Ehr.).
<table>
<thead>
<tr>
<th>concentrations</th>
<th>plant species</th>
<th>measurements</th>
<th>effects</th>
<th>reference</th>
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</thead>
<tbody>
<tr>
<td>25 µg g⁻¹ (50%)</td>
<td>grasses</td>
<td>root length</td>
<td>no effect</td>
<td>Ingham et al., 1986</td>
</tr>
<tr>
<td>a.i.)</td>
<td></td>
<td>live shoot dry weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 µg g⁻¹ (50%)</td>
<td>blue grama</td>
<td>live shoots</td>
<td>no effect</td>
<td>Ingham and Coleman 1986</td>
</tr>
<tr>
<td>a.i.)</td>
<td></td>
<td>dead shoots</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>total root length</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>new roots length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.5 g m⁻² (47%)</td>
<td>sorghum</td>
<td>crop biomass</td>
<td>no effect in</td>
<td>Hu et al., 1995</td>
</tr>
<tr>
<td>a.i.)</td>
<td></td>
<td>grain biomass</td>
<td>the 1st year,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>but increase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>in the 2nd</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>year</td>
<td></td>
</tr>
<tr>
<td>250 and 500 mg</td>
<td>white clover</td>
<td>dry weight and N content</td>
<td>increase but</td>
<td>Fisher 1976</td>
</tr>
<tr>
<td>kg⁻¹</td>
<td></td>
<td></td>
<td>NS¹</td>
<td></td>
</tr>
<tr>
<td>0.01-100 mg l⁻¹</td>
<td>watermelon 'Starbrite'</td>
<td>No. of leaves leaf, stem and shoot dry weight, whole plant dry weight</td>
<td>NS</td>
<td>Liu et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td>longleaf pine</td>
<td>top weight</td>
<td>increase but</td>
<td>Pawuk et al., 1980</td>
</tr>
<tr>
<td>a.i./seedling in</td>
<td></td>
<td>root weight</td>
<td>NS²</td>
<td></td>
</tr>
<tr>
<td>50 ml water</td>
<td></td>
<td>stem diameter</td>
<td></td>
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</tr>
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</table>

Table 1.13: Effects of captan on plant growth.
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Species</th>
<th>Response</th>
<th>Author and Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg a.i./seedling in 50 ml water</td>
<td>Longleaf pine</td>
<td>Top weight, root weight, stem diameter</td>
<td>Pawauk et al., 1980</td>
</tr>
<tr>
<td>N/A</td>
<td>Monterey pine</td>
<td>Development of ectomycorrhizae inhibition</td>
<td>Theodorou and Skinner 1976 in</td>
</tr>
<tr>
<td>N/A</td>
<td>Caribbean pine</td>
<td>No. of seedlings, total amount of ectomycorrhizae on seedlings increase</td>
<td>Hong 1976 in</td>
</tr>
<tr>
<td>25 µg g⁻¹</td>
<td>Douglas fir</td>
<td>Survival odds, dry weight of shoot, root and needle, basal area increment, seedling leader growth, No. of short roots increase</td>
<td>Colinas et al., 1994</td>
</tr>
<tr>
<td>25 µg g⁻¹</td>
<td>Douglas fir</td>
<td>Survival odds, dry weight of shoot, root and needle, basal area increment, seedling leader growth, No. of short roots increase except basal area increment</td>
<td>Colinas et al., 1994</td>
</tr>
</tbody>
</table>

[to be continued]
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Plant Type</th>
<th>Effect</th>
<th>Percentage Increase/Decrease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 μg g⁻¹</td>
<td>Douglas fir</td>
<td>survival odds, dry weight of shoot, root and needle, basal area increment, seedling leader growth, No. of short roots</td>
<td>increase or no effect except significant effects on survival odds⁶</td>
<td>Colinas et al., 1994</td>
</tr>
<tr>
<td>1 mg pot⁻¹</td>
<td>tomato</td>
<td>root/shoot ratio</td>
<td>no effect</td>
<td>Somda et al., 1991</td>
</tr>
<tr>
<td>25 mg kg⁻¹</td>
<td>tomato</td>
<td>total dry weight</td>
<td>increase</td>
<td>Somda et al., 1990</td>
</tr>
<tr>
<td>N/A</td>
<td>apple</td>
<td>yield and fruit quality</td>
<td>increase</td>
<td>Ross 1964</td>
</tr>
<tr>
<td>1.5 mg a.i./100 ml onion</td>
<td>diameters of bulbs and dry matter</td>
<td>decrease</td>
<td>De Bertoldi et al., 1977, 1978</td>
<td></td>
</tr>
</tbody>
</table>

1. NS means not significant compared to the untreated controls.
2. Longleaf pine seedlings were infested with *Pisolithus tinctorius*.
3. Longleaf pine seedlings were not infested with *Pisolithus tinctorius* but were naturally infested by airborne fungi (mostly *Thelephora terrestris* Ehr.).
4. Measurements were taken at Cedar Camp clearcut.
5. Measurements were taken at matur forest of Douglas fir adjacent to Cedar Camp clearcut.
6. Measurements were taken at vigorous plantation of Douglas fir.
RESEARCH OBJECTIVES:

The main purpose of this proposal is to establish a standardized methodology for evaluating the impact of fungicides, and to investigate the effects of three fungicides, benomyl, captan and chlorothalonil on soil microbial processes and nitrogen dynamics in the soil with different qualities of organic substrates, such as ground alfalfa and wheat straw, and the soil with distinct properties (silt loam vs. sandy loam) by three steps, long-term batch incubations in the laboratory, integrated plant-soil microcosm technique in greenhouse and in field condition. My main objectives are listed below:

1. To examine the effects of fungicides on soil microbial processes and nitrogen dynamics in batch incubations in the laboratory.
2. To develop and test an integrated microcosm technique for evaluating the effects of fungicides on soil microbial processes, nitrogen transformations, litter decomposition and plant growth under three organic amendments.
3. To determine the effects of fungicides on soil microbial activity, nitrogen dynamics, litter decomposition and nutrient availability of plants under two different types of soils.
4. To examine and verify the effects of fungicides on soil microbial activity, nitrogen transformations, litter decomposition and plant growth under field conditions.
5. To develop an integrated approach for evaluating the impact of pesticides (for example, fungicides) on important soil ecological processes and plant growth.

HYPOTHESES AND EXPECTED RESULTS

1. Fungicides affect soil microbial processes by reducing soil respiration rates and decreasing enzyme activities because of the reduction of fungal population, the major portion of soil microorganisms, and negative influences of other vulnerable soil microorganisms.
2. Fungicides affect soil nitrogen transformations resulting from the inhibition of soil nitrification, the promotion of soil ammonification, the change of microbial biomass N content in soil, and the alteration of net rates of N mineralization and immobilization.

3. Litter decomposition in soil is influenced by fungicide applications because of the changes of decomposer communities, functional diversity of soil microorganisms and affecting microbial activities.

4. Fungicides affect nutrient availability to plants due to the change of net N mineralization and immobilization and different amount of trace elements present in soils.

5. Soils with different organic matter contents may affect adsorption/absorption of fungicides onto soil particles and strengthen/lessen the effects of the fungicides on soil microbial activity and ecological processes.

6. Soils with distinct physical and chemical properties may affect behaviors of fungicides in the soils and directly or indirectly influence soil microbial processes.

7. An integrated microcosm technique can become a valid and ecotoxicological approach to evaluate the effects of pesticides (fungicides) on soil ecological processes.

8. Results from the field study should within acceptable limits, agree with outcomes of microcosm studies.
CHAPTER 2

EFFECTS OF THE FUNGICIDES BENOMYL, CAPTAN AND CHLOROTHALONIL ON SOIL MICROBIAL ACTIVITY AND NITROGEN DYNAMICS: LABORATORY BATCH INCUBATIONS

SUMMARY

The use of fungicides in agriculture to protect plants from pathogens is very common. However, there is a general lack of information on the overall side-effects of fungicides on soil ecological processes. We examined the effects of three fungicides, benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate), captan (N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) and chlorothalonil (tetrachloroisophthalonitrile), on soil microbial activity (substrate-induced respiration and dehydrogenase activity), and nitrogen dynamics. Soil was treated with the fungicides at approximate field application rates (benomyl, 51 mg kg⁻¹, captan, 125 mg kg⁻¹ and chlorothalonil, 37 mg kg⁻¹, active ingredient) and incubated at 30°C in the laboratory for 56 days. The soil was amended with, either ground alfalfa leaves or ground wheat straw, to provide additional nutrients for soil microorganisms and to alter rates of nitrogen mineralization/immobilization. All three fungicides suppressed peak respiration in the unamended soil 14 days after treatment, but had different effects in the amended soils. Soil dehydrogenase activity (DHA) was generally stimulated by benomyl and chlorothalonil, but decreased by captan in the straw-amended and unamended soils. Overall, the fungicide-treated soils had lower microbial biomass N (BION) concentrations than
untreated soils. Captan-treated soils had much higher NH₄-N concentrations than the control soils with or without the organic amendments; benomyl and chlorothalonil had little influence on soil NH₄-N concentrations. Net N mineralization and nitrification rates were influenced by the fungicide treatments as well as by the addition of organic materials. Captan-treated soils had significantly higher N mineralization rates than the untreated soils. Chlorothalonil-treated soils had a similar pattern to captan but reached the peak nitrification rates earlier, on day 7, in the alfalfa-amended soil. The effects of fungicides on soil microbial activity and nitrogen dynamics depended on the quality of the organic materials in the soil. Patterns of response of soil nutrient cycling processes were specific to each of the fungicide. Captan appeared to have more pronounced effects on soil microbial activity and nitrogen dynamics than benomyl or chlorothalonil, at typical field application rates.

**Key words:** fungicides, benomyl, captan, chlorothalonil, soil microbial activity, nitrogen transformation

**INTRODUCTION**

The number of reports related to the effects of pesticides on non-target soil organisms is enormous (Edwards, 1988). However, compared with those concerned with the effects of herbicides or insecticides, many of the reports of the effects of fungicides on soil organisms and processes are very limited and dated, with the majority published in the 1970s and 1980s (Agnihotri, 1970; Houseworth and Tweedy, 1973; Kreytzer, 1963, Prasad et al., 1971). Most of these studies were done under laboratory conditions and for relatively short time periods (Wainwright and Pugh, 1973, 1974). Recently, a few research groups have looked at the effects of fungicides on soil ecosystems by removing or reducing component groups of the soil food web (Hu et al., 1995; Ingham et al., 1991).

Like other pesticides, fungicides are bioactive chemicals which interfere not only with the biochemical and physiological reactions of target plant pathogens, but may also influence other non-target microorganisms in soils (Tu, 1993). Fungicides, such as
benomyl, captan and chlorothalonil are considered to be non-selective and are commonly used to control a wide spectrum of plant diseases and therefore are most likely to cause critical changes in soil microbial populations. It has been concluded that even a single application at field rates is likely to cause changes in nitrogen transformations and availability in soils (Anderson et al., 1981). Many researchers have studied the effects of benomyl, captan or chlorothalonil on either soil microbial activity or specific soil nitrogen transformations in the short term, but little research has been published on the longer-term effects of the fungicides on soil microbial processes and nitrogen dynamics or on potential interaction with soil organic materials of different qualities.

Our main objective in this study was to investigate the system-level impacts of three fungicides (benomyl, captan and chlorothalonil) in soils with different organic amendments (alfalfa and wheat straw), on soil microbial activity and nitrogen dynamics, in a 56-day laboratory incubation. Specifically, we wished to test the hypothesis that each fungicide had specific influences on soil microbial activity and nitrogen transformations. In addition, it is possible that amendments of different qualities of organic materials could either accelerate or reduce the impacts of these fungicides on soil microbial processes

MATERIALS AND METHODS

Soil Incubation

This study consisted of two separate soil incubations. Captan (50% wettable powder) and chlorothalonil (29.6% Daconil 2787®) were used in the first incubation and benomyl (50% wettable powder) was tested in the second incubation. The soil used for both incubations was a Canfield silt-loam Luvisol (fine, mixed, mesic Typic Fragiudalf), collected from the surface 15-20 cm of soil in agricultural research plots in Wooster, Ohio. It had a pH (H₂O) of 6.3, an organic matter content of 4%, total C 2.3% and total N 0.2%. Two organic amendments, alfalfa leaves (43.4% C, 4.88% N, C/N = 8.9), and wheat straw (44.7% C, 0.53% N, C/N = 84) were used in the experiment. Both materials were oven dried (60 °C) and ground in a centrifugal mill (200 μm mesh) before addition to the soil at a ratio of approximately 50:1 (soil:amendment, dry wt basis). The soils were
coarsely-sieved (6 mm) and mixed with the organic amendments and fungicides by misting aqueous suspensions of the fungicides to obtain a moisture content of 20%. Soil concentrations of benomyl, captan and chlorothalonil that were applied approximately their field application rates (51 mg kg\(^{-1}\) a.i. for benomyl, 125 mg kg\(^{-1}\) a.i. for captan and 37 mg kg\(^{-1}\) a.i. for chlorothalonil). To calculate these values, we assumed a soil bulk density of 1.2 g cm\(^{-3}\) and an effective soil depth of 2 cm, since the fungicides are typically surface-applied. Fifty grams of amended + fungicide-treated soils were incubated in 150 ml (5 oz.) plastic containers at 30 °C for 56 days. There were four replicate cups for each treatment combination. Three soil amendments were made: (1) unamended control soil; (2) alfalfa-amended soil; (3) wheat straw-amended soil. Control soils received only equal amounts of deionized water. After 3, 7, 14, 28, and 56 days, the soils were processed and analyzed for microbial activity, using substrate induced respiration (SIR) and soil dehydrogenase activity (DHA) as indices, and N pools, including concentrations of extractable inorganic N (NH\(_4\)-N and NO\(_3\)-N), microbial biomass N (BION), dissolved organic N (DON) and net rates of N mineralization and nitrification.

**Soil sampling and analysis**

Substrate induced respiration (SIR), an index of soil microbial biomass and activity (Parkinson and Coleman, 1991), was determined as the rate of evolution of CO\(_2\) from soil to which a readily assimilable substrate (glucose) was added. Twenty-five grams of soil were amended with 5 ml glucose solution (32 mg ml\(^{-1}\)) and CO\(_2\) was trapped in alkaline solution (20 ml 0.02N NaOH). The soil and CO\(_2\) traps were incubated in sealed wide-mouth 32 oz. Ball jars for 6 hours at room temperature. The NaOH solutions were treated with excess 3N BaCl\(_2\) solution and titrated with a 0.02 N HCl solution. Phenolphthalein was used as the indicator of the titration end point. Soil dehydrogenase activity (DHA) was measured as the reduction of TTC (2,3,5-triphenyl tetrazolium chloride) to triphenyl tetrazolium formazn (TPF) during 6 hr incubations of 1 g samples at 40 °C, with additions of 8 mg ml\(^{-1}\) glucose in buffer solution, using a modification of the method of Casida (1977). After incubation, tubes were extracted with 10 ml buffer-methanol solution and
formation of TPF was determined on a Lachat autoanalyzer with a 480 nm filter (Subler et al., 1997).

Concentrations of extractable inorganic N (NH₄-N, NO₃-N) were determined in 0.5 M K₂SO₄ extracts (1:5 soil:extractant) using the phenate and cadmium reduction/diazotization methods with a Lachat AE flow-injection autoanalyzer. Dissolved organic N (DON) concentrations were calculated as the difference between the initial total extractable inorganic N concentration and the NO₃-N concentration determined after alkaline persulfate digestion of the soil extracts (Cabrera and Beare, 1993). Microbial biomass N (BION) was determined using the chloroform fumigation-direct extraction method (Brookes et al. 1985). Net N mineralization (NMIN) was calculated as the difference from the soil inorganic N (NH₄-N + NO₃-N) concentrations between two sampling dates. Net nitrification (NITR) was calculated as the difference from the soil NO₃-N concentrations between two sampling dates.

**Statistical analysis**

Due to relatively few replications in the experiment, the data were rank-transformed before being analysed to equalize error variance and, given the small sample size, to provide a potentially more robust method than traditional parametric methods (Conover and Iman, 1981). For each incubation, a two-way analysis of variance (ANOVA) was used to determine the significance of effects of each fungicide and for the interactions between fungicide and amendment treatments. For dependent variables that were affected significantly by treatments, Tukey's multiple comparison was used for separation of means. Correlation analysis was used to explore relationships among variables. Significance was defined as P ≤ 0.05, unless otherwise indicated. SAS statistical software was used for all analyses (Statistical Analysis Software Inc., 1990). Figures and tables present means and standard errors of untransformed data.
RESULTS

The effects of benomyl on soil microbial activity and nitrogen dynamics

Benomyl increased peak microbial activity (SIR and DHA), which occurred in the first week, in the unamended and alfalfa-amended soils. Benomyl also significantly increased microbial biomass during the first week in the same soils (Fig. 2.1). On the other hand, microbial activity and biomass were reduced by benomyl in the straw-amended soils. Benomyl generally increased soil NH₄-N and NO₃-N concentrations in all soils, especially in the alfalfa-amended soils, and the effects were significant for the first 2 weeks. Peak DON concentrations were also increased by the benomyl treatment in the alfalfa-amended soil (Fig. 2.3). Initial mineralization and nitrification rates were enhanced by benomyl in the straw-amended and unamended soils but, after two weeks, both rates were declined compared to the controls (Table 2.1).

The effects of captan on soil microbial activity and nitrogen dynamics

Captan had varied effects on microbial activity and biomass in the differently-amended soils. Captan reduced peak microbial activity and biomass during the first two weeks in the unamended soil. In the alfalfa-amended soil, captan increased microbial activity initially but subsequently reduced activity and biomass. On the other hand, in the straw-amended soil, captan increased SIR throughout the incubation, but generally reduced DHA and BION (Fig. 2.2). Captan-treated soils had significantly higher NH₄-N concentrations than the untreated controls for all soils. Captan generally reduced NO₃-N concentrations to some extent in all three soils. DON concentrations were increased significantly by the captan treatment in the amended soils (Fig. 2.4). Net N mineralization and nitrification rates varied among the sampling dates. General trends found were that mineralization and nitrification were reduced initially by captan, then followed by significant increases in the unamended and straw-amended soils. In the alfalfa-amended soil, captan inhibited nitrification significantly for the first two weeks (Table 2.2).
The effects of chlorothalonil on soil microbial activity and nitrogen dynamics

The effects of chlorothalonil on microbial activity and biomass were not as pronounced as those of the other two fungicides. Chlorothalonil generally inhibited peak microbial activity and biomass to some extent in all three soils (Fig. 2.2). Chlorothalonil had no effect on $\text{NH}_4^+$-N concentrations in any of the soils tested. Chlorothalonil caused a large increase in $\text{NO}_3^-$-N concentrations significantly for the first two weeks in the unamended soil and for all dates in the straw-amended soil; however, in the alfalfa-amended soil, chlorothalonil reduced $\text{NO}_3^-$-N significantly. Chlorothalonil increased DON concentrations slightly one month after the treatment in the straw-amended soil, but not in the other two soils (Fig. 2.4). Chlorothalonil significantly reduced mineralization rates for the first two weeks in all three soils. Nitrification rates were increased by chlorothalonil in the unamended soil which resulted in higher concentrations of $\text{NO}_3^-$-N in the soil (Table 2.3).
Figure 2.1: Effects of benomyl on soil substrate induced respiration, soil dehydrogenase activity and microbial biomass N concentrations in three soils during the 56-day incubations. Soil treatments were unamended, alfalfa-amended and wheat straw-amended soils. Significant differences at P≤ 0.05 and 0.10 are indicated by * and +, respectively.
Figure 2.2: Effects of captan and chlorothalonil on soil substrate induced respiration, soil dehydrogenase activity and microbial biomass N concentrations in three soils during the 56-day incubations. Soil treatments were unamended, alfalfa-amended and wheat straw-amended soils. Significant differences at P ≤ 0.05 and 0.10 are indicated by * and +, respectively.
Figure 2.3. Effects of benomyl on soil ammonium nitrogen, nitrate nitrogen and dissolved organic N concentrations in three soils during the 56-day incubations. Soil treatments were unamended, alfalfa-amended and wheat straw-amended soils. Significant differences at P ≤ 0.05 and 0.10 are indicated by * and +, respectively.
Figure 2.4. Effects of captan and chlorothalonil on soil ammonium nitrogen, nitrate nitrogen and dissolved organic N concentrations in three soils during the 56-day incubations. Soil treatments were unamended, alfalfa-amended and wheat straw-amended soils. Significant differences at P ≤ 0.05 and 0.10 are indicated by * and +, respectively.
<table>
<thead>
<tr>
<th>Amendments</th>
<th>Time (days)</th>
<th>Fungicides</th>
<th>Net N Mineralization Rates (mg N kg⁻¹ soil day⁻¹)</th>
<th>Net Nitrification Rates (mg N kg⁻¹ soil day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>control</td>
<td>benomyl</td>
<td>control</td>
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<td>56</td>
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<td>1.46±0.234</td>
<td>1.07±0.093</td>
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<td>Alfalfa-amended</td>
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</tr>
<tr>
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<tr>
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<td>0.01±0.107</td>
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<td></td>
<td>56</td>
<td>-0.11±0.033</td>
<td>0.037±0.009</td>
<td>-0.13±0.031</td>
</tr>
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</table>

Significant differences at P ≤ 0.05 are indicated by *. 

Table 2.1: Effects of benomyl on soil net N mineralization and net nitrification rates.
<table>
<thead>
<tr>
<th>Amendments</th>
<th>Time (days)</th>
<th>Fungicides</th>
<th>Net N Mineralization Rates (mg N kg(^{-1}) soil day(^{-1}))</th>
<th>Fungicides</th>
<th>Net Nitrification Rates (mg N kg(^{-1}) soil day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unamended</td>
<td>7</td>
<td>control</td>
<td>0.111±0.3401</td>
<td>captan</td>
<td>-0.107±0.1200*</td>
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<tr>
<td></td>
<td>14</td>
<td>control</td>
<td>0.008±0.0699</td>
<td>captan</td>
<td>1.592±0.1783*</td>
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<tr>
<td></td>
<td>28</td>
<td>control</td>
<td>0.002±0.0166</td>
<td>captan</td>
<td>0.544±0.3128</td>
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<td>56</td>
<td>control</td>
<td>0.001±0.0035</td>
<td>captan</td>
<td>-0.899±0.1408*</td>
</tr>
<tr>
<td>Alfalfa-amended</td>
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<td>control</td>
<td>-14.999±2.2362</td>
<td>captan</td>
<td>-1.551±2.3674*</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>control</td>
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<td>captan</td>
<td>2.999±1.1545*</td>
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<tr>
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<td>28</td>
<td>control</td>
<td>0.003±0.0027</td>
<td>captan</td>
<td>-7.866±0.3119*</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>control</td>
<td>0.223±0.0825</td>
<td>captan</td>
<td>-0.020±0.0468</td>
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<tr>
<td>Straw-amended</td>
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<td>captan</td>
<td>-1.334±0.0230*</td>
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<td>captan</td>
<td>0.313±0.1163*</td>
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<td>control</td>
<td>0.013±0.0075</td>
<td>captan</td>
<td>0.181±0.0447</td>
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<td>56</td>
<td>control</td>
<td>0.042±0.0260</td>
<td>captan</td>
<td>-0.087±0.0347*</td>
</tr>
</tbody>
</table>

Significant differences at P≤ 0.05 and 0.10 are indicated by * and +, respectively.

Table 2.2: Effects of captan on soil net N mineralization and nitrification rates.
<table>
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<tr>
<th>Amendments</th>
<th>Time (days)</th>
<th>Fungicides</th>
<th>Net N Mineralization Rates (mg N kg(^{-1}) soil day(^{-1}))</th>
<th>Fungicides</th>
<th>Net Nitrification Rates (mg N kg(^{-1}) soil day(^{-1}))</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>chlorothalonil</td>
<td>control</td>
<td>chlorothalonil</td>
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<td>0.807±0.3364</td>
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<td>0.623±0.2589</td>
<td>-0.382±0.3296*</td>
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<td>0.042±0.0181*</td>
<td>0.662±0.2369</td>
<td>0.525±0.0221*</td>
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<td>Alfalfa-amended</td>
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<td>-14.999±2.2362</td>
<td>-15.34±1.5637*</td>
<td>15.00±3.4394</td>
<td>10.84±1.7526*</td>
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<td>14</td>
<td>-3.26±0.9521</td>
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<td>4.01±1.5311*</td>
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<td>0.22±0.0825</td>
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<td>13.41±4.0986</td>
<td>8.17±1.8921*</td>
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<td>Straw-amended</td>
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<td>-1.23±0.0519*</td>
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<td>0.01±0.0064</td>
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<td>-0.13±0.1337</td>
<td>0.003±0.0031</td>
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</table>

Significant differences at P≤ 0.05 and 0.10 are indicated by * and +, respectively.

Table 2.3: Effects of chlorothalonil on soil net N mineralization and nitrification rates.
DISCUSSION

The application of fungicides for the control of plant diseases has become an essential part of crop production in many parts of the world. However, current knowledge of environmental risks posed by such xenobiotics is most advanced for aquatic ecosystems and there is still a need to characterize the best biological indicators for the soil environment (Peichl and Reiml, 1990). Reproducible data can be best obtained under standardized condition of a laboratory test, especially when it is related to soil microbial transformations (Schuster and Schröder 1990). Studying soil microbial activity can provide insight into biochemical processes in soils and is sensitive as a biological index (Frankenberger and Dick. 1983). Using microbial activity and nitrogen dynamics as indices to understand the impacts of fungicides on soil ecosystems better, may be helpful in developing a strategy to apply fungicides to control plant diseases and improving plant health with minimal environmental hazards.

The influence of fungicides on soil microorganisms and microbial processes in soil depends on many factors. Major factors include physical, chemical and biochemical conditions in the soil, and the nature and concentration of the fungicides (Vyas, 1988). In our study, the effects of the fungicides on patterns of soil microbial activity (SIR and DHA) were dependent on the specific fungicide and the quality of organic materials in the soil. Soil respiration rates were reduced by captan and chlrothalonil, primarily during the first 14 days, and then the rates started rising toward the end of the incubation (Fig. 2.1). Domsch (1964) reported that, after application of soil fungicides, soil respiration was inhibited for a short time after application, but the surviving microflora recovered soon and the respiration rate rose to high levels, exceeding even those in the untreated soils. Such phenomena were based not only on the return of the original microbial population, but to the increased activity of a few resistant species (Domsch, 1970), or based on microbial utilization of fungicides as a substrate rather than stimulation of the population (Vyas, 1988).

The literature about the effects of chlorothalonil on soil respiration rates were too sparse to make any appropriate overall conclusions. Some researchers reported that the environmental impact of chlorothalonil was too small to be detected (Naganawa et al,
1989), others found that in chlorothalonil-treated sandy loam oxygen consumption increased significantly after 96 hours of incubation (Tu 1993). However, in our study, chlorothalonil caused a significant reduction of SIR in the unamended and alfalfa-amended soils. In the case of benomyl, significant reductions (P < 0.10) of SIR was observed by day 14, in the unamended soil, then the rate soon recovered to the same level as in the controls. Smiley and Craven (1979) found that numbers of fungi appeared to be reduced by the benzimidazole-derivatives such as benomyl, but the reduction was only temporary (Simon-Sylvestre and Fournier, 1979).

Addition of organic materials to the soil could change the microbial populations and composition in the soil. Our results showed that higher microbial activity and biomass occurred in the amended soils. Zelles et al (1985) reported that soil respiration was enhanced considerably by the addition of 0.5% alfalfa meal, but that also depended on the nature and concentrations of the fungicide. In our study, we used a normal field application rate of captan (125 mg kg\(^{-1}\), a.i.) which caused a reversible inhibition of SIR.

Another important index of soil microbial activity that we measured was the soil dehydrogenase activity. In our study, DHA showed different patterns in response to the fungicide as did SIR (Fig. 2.1 and 2.2). DHA increased during the first 7 days then gradually reached a plateau by the end of the experiment. Although positive correlation between DHA and the rates of respiration (perhaps related to degradation) have been found in some soils (Casida et al., 1964, Parkinson and Coleman, 1991). In the present study, we did not find such a correlation. However, a strong correlation was obtained between DHA and BION (\(r^2 = 0.83\)), which might indicate that DHA was a good index for soil microbial biomass, which was primarily nonfungal biomass. Ross (1973) found significant correlation between DHA, \(O_2\) uptake and numbers of aerobic bacteria. Although we did not quantify the numbers of bacteria, fungi and other microbes in the soils, significant reductions in SIR provided circumstantial evidence that the soil fungi were being influenced by the fungicide treatments. Houseworth and Tweedy (1973) reported that soil fungal populations were decreased by treatment with 10 mg kg\(^{-1}\) captan after 2, 16, and 44 days and that the bacterial populations were higher after the captan
treatment. The increase of bacterial populations probably resulted from a lack of competition from those fungi that were inhibited by fungicides. The numbers of fungi and heterotrophic bacteria increased 28 days after the benomyl treatment, and the flush of bacteria which followed the application of the fungicides was probably responsible for many of the biochemical changes that occurred (Wainwright and Pugh, 1974). The amended soils had a higher DHA compared to the unamended soil, which indicated that added organic materials stimulated certain portion of soil microbial community strongly, in order to enhance the dehydrogenase activity.

In our study, the dynamics of the soil NH$_4$-N, NO$_3$-N, DON (Fig. 2.3 and 2.4), and BION concentrations (Fig. 2.1 and 2.2), net N mineralization and nitrification rates (Table 2.1, 2.2 and 2.3) were all influenced by the fungicide treatments and the different quality of organic materials. Total inorganic N (NH$_4$-N and NO$_3$-N) concentrations were increased significantly by the fungicides, possibly due to higher rates of mineralization, which resulted in higher NH$_4$-N concentration in soils. Our results indicated that higher NH$_4$-N concentrations released in captan-treated soils and higher mineralization and nitrification rates, resulted from the mineralization of killed organisms. However, Agnihotri (1971) reported that ammonification was not affected by captan at 40 and 60 mg kg$^{-1}$ because the NH$_4$-N accumulated that might have been due to the liberation of ammonia from organic residues of the affected microflora. Changes caused by captan treatments have been discussed through differential effects of the fungicide on the soil microbial community (Wainwright and Pugh, 1973). On the other hand, applications of fungicides at normal rates retarded nitrification significantly (Prasad et al., 1971) and the reduction of NO$_3$-N and increase of NH$_4$-N concentrations in our study were probably due to the nitrifiers being susceptible to the fungicides (Kreytzer, 1963, Vyas, 1988), or by altering the ratio and numbers of fungi and heterotrophic bacteria in soils (Wainwright and Pugh, 1974). Soil nitrifiers are a very specialized group of microorganisms restricted to a few genera of bacteria (Wainwright and Pugh, 1973). Soil bacteria Nitrosomonas sp. and Nitrobacter sp., responsible for oxidation of ammonium to nitrate via nitrite (nitrification),
have been reported as being among the most susceptible to pesticides, but results of experiments of this kind are often contradictory (Törnstensson and Wessen, 1984).

Tu (1993) reported that chlorothalonil did not influence nitrification compared to that in the control soils. Chlorothalonil enhanced the net mineralization rates in the unamended and straw-amended soils and reduced nitrification rates in the unamended and alfalfa-amended soils. The $\text{NH}_4$-N and $\text{NO}_3$-N concentrations in the alfalfa-amended soil were much higher than in the other two soils, which might indicate that the ground alfalfa leaves offered readily available N for soil microorganisms, to accelerate N mineralization, even if a part of soil microorganisms were inhibited or killed by the fungicides. In our study, benomyl did not affect mineralization significantly but caused significant effects on nitrification after days 7 and 14 in the alfalfa-amended soil (Table 2.1). Contradictory results reported in the literature have indicated that benomyl can inhibit nitrification (Hofer et al., 1971, Wainwright and Pugh, 1974), increase in rates of nitrate accumulation (Törnstensson and Wessen, 1984, Van Faassen, 1974), or have no influence in response to fungicides (Helweg, 1973). A very high concentration of benomyl (1000 mg kg$^{-1}$) inhibited the oxidation of ammonium to nitrate drastically and this inhibitory action was well-pronounced even after 30 days (Ramakrishna et al., 1979). In our study, the changes in the net N mineralization and nitrification rates were dependent on the quality of organic amendments and temporal basis.

CONCLUSIONS

In the present study, we were able to determine some ecological side-effects of fungicides on soil microbial activity and nitrogen dynamics. The results indicate that the impact of fungicides on soil systems depended on the different quality of organic materials and nature of the fungicides. In general, all three fungicides had same inhibitory effects on soil microbial activity and biomass, but soil dehydrogenase activity was increased by the fungicide treatments. Fungicides can inhibit as well as stimulate certain groups of microorganisms in the soil. Due to these changes, the influences of fungicides on different nitrogen pools depend on the nature and properties of the fungicides. Many of the
observed changes in nitrogen dynamics in our study suggest that the addition of organic materials can change the impact of fungicides on soil systems. Each fungicide that was influenced by organic amendments, exhibited different effects on soil processes. In comparison to benomyl and chlorothalonil, captan appeared to have much more profound effects on both soil microbial activity and nitrogen dynamics. Additional research is needed to determine if the effects observed in these small-scale soil incubations can be extrapolated to those in larger more realistic systems such as greenhouse and field studies that include growing plants. Nevertheless, this study provides strong evidence that agricultural practices (e.g. organic amendments in our study) can affect the impact of fungicides on soil ecosystems significantly, even when the fungicides are applied to soil at the normal application rates.
CHAPTER 3

A MICRO COSM APPROACH FOR EVALUATING THE EFFECTS OF FUNGICIDES, BENOMYL AND CAPTAN, ON SOIL ECOLOGICAL PROCESSES AND PLANT GROWTH: COMPARISONS OF DIFFERENT ORGANIC AMENDMENTS

SUMMARY

In this study, two broad-spectrum fungicides, benomyl and captan, commonly used in current agricultural systems, were chosen to assess the impact on soil ecological processes and to establish the standardized evaluating approaches. A greenhouse microcosm approach was carried out to evaluate the effects of two fungicides on soil ecological processes by measuring: (1) soil microbial activity and biomass, including soil substrate-induced respiration, soil enzyme activity (dehydrogenase, urease and acid phosphatase) and microbial biomass nitrogen concentrations, (2) nitrogen transformations, including extractable inorganic nitrogen, dissolved organic nitrogen concentrations, net N mineralization and nitrification rates, (3) organic matter (litter) decomposition, (4) leaching processes (ion-exchange resin bags) and (5) plant growth. Each microcosm consisted of freshly-collected field soil, which was coarsely-sieved, mixed and packed gently into a 5 cm x 15 cm high density polyethylene plastic cylinder. The effects of the fungicides on soil processes were assessed at frequent intervals after amendment of soil with different organic materials. The quality of the organic matter strongly influenced the effects of two fungicides on soil microbial processes and nitrogen availability. Substrate-induced respiration, soil enzyme activities (except urease activity), microbial biomass N

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and dissolved organic N concentrations were decreased significantly by the fungicide treatment. Soil urease activity, NH$_4^-$-N and NO$_3^-$-N concentrations, and initial net N mineralization and nitrification rates were increased by the fungicide treatment. Wheat straw decomposition rates were also inhibited by the fungicide application. Leaching patterns of NH$_4^-$-N and NO$_3^-$-N in ion-exchange resin bags varied with fungicide treatments. Captan increases amounts of NH$_4^-$-N and NO$_3^-$-N in the resin bags significantly compared to the untreated controls or in benomyl-treated soils. The germination success of wheat seeds after 7 days, plant biomass (shoot + roots) as well as total nitrogen uptake were all enhanced by captan. The two fungicides differed in their effects on soil processes and plant growth, as well as an nitrogen uptake by plants, with captan having a greater and longer-lasting influence than benomyl. Taken together, we conclude that the techniques and consequent data provided not only an accurate evaluation of the impact of fungicides on soil processes, but also a better understanding of the interactions between applied fungicides and soil ecological processes and an idea how this interaction affects nutrient cycling and soil fertility in agricultural systems.

**Keywords:** Soil Microcosm, Fungicides, Benomyl, Captan, Microbial activity, Enzyme activity, Soil nitrogen, Litter decomposition, Ion-exchange resin, $^{15}$N-labeled ammonium sulfate

**INTRODUCTION**

Pesticides, including fungicides, have the potential to kill or affect the function of a diverse range of soil organisms that contribute to soil biological processes and maintain soil structure and fertility (e.g. Edwards, 1988; Edwards and Bater, 1990; Vyas, 1988). It is important to avoid contamination of soils by chemicals that have deleterious effects on soil processes and thereby affect soil health, in order to maintain soil fertility. The fertility of soil, i.e. its capacity to produce acceptable crops, depends not only on its physical constitution and its nutrient content, but also on the intensity of the biological processes that take place within it. The soil although a dynamic system, appears to be in a biological
equilibrium, but this equilibrium is a precarious one, since any disturbance of the environment presents the potential to modify the numbers and activity of the microflora and consequently the soil's fertility. The increasing usage of pesticides, although intended to protect crops, may alter this equilibrium, by direct or indirect action, after a short, average, or long period of time, depending on whether the product acts quickly or persists longer in its initial state or metabolic forms (Simon-Sylvestre and Fournier, 1979).

Harmonized methods which can accurately evaluate the impact of pesticides on soil microorganisms, which play an essential role in soil biological processes, including nitrogen transformation, organic matter decomposition and nutrient availability, are necessary. Although a number of studies on the effects of pesticides, mainly herbicides and insecticides on soil processes, have been reported, very little research was done to examine the long-term effects of fungicides on these soil microbial activities and overall ecological processes, which are related to soil fertility. In addition, the impact of fungicides on soil processes, based on interrelationships within microbial populations and community- or system-level interactions are also poorly understood.

The literature on the effects of chemicals such as pesticides on soil organisms and soil processes is extremely diverse, ranging from reports of the effects of chemicals on individual species of organisms, to those on overall populations of organisms and on individual biological systems using a great diversity of methods in both field and laboratory (Edwards and Bater, 1990). In addition, very little research has been done to examine the longer-term impact of fungicides on soil microbial activity and ecological processes (Tu, 1993) and little information is available to assess the effects of fungicides in integrated microcosm studies (Takagi et al., 1991). Moreover, many experiments have been done in vitro, involved unrealistic doses of chemicals, methods of application and modes of exposure, so it is impossible to compare the results of experiments by different workers, using the same chemicals, and to reach a valid conclusion. Registration authorities have an urgent need for standardized or harmonized methodologies that will assess adequately the potential hazard of any pesticides in soil ecosystems. Hence, there is an urgent need for integrated, innovative testing methodologies involving testing the effects of pesticides on important ecological processes in soils (Edwards, 1988).
In recent years, interest has developed in the use of microcosm technique for ecotoxicological assays in an laboratory and elsewhere (Bogomolov et al., 1996; Draggan, 1977; Edwards et al., 1994; Gillett, 1989; Jackson et al., 1977; O'Neill et al., 1977; Parmelee et al., 1993; Sheppard 1994; Van Voris et al., 1985). Such microcosms have usually consisted of soil units containing multiple biotic species, and have ranged in size from a few grams of soil to cores as large as a meter in diameter. We consider that a microcosm approach can assess the impact of contaminants at different levels of biological organization and produce data that is more relevant to field situations. Moreover, they have advantages of being relatively simple, inexpensive and extremely reproducible.

In the present study, we adopted the methodology of Edwards et al. (1994), adding different quality of organic materials (ground alfalfa leaves and wheat straw) and \( \text{NH}_4\text{SO}_4 \). Our main objective was to evaluate the usefulness of the microcosm approach and to show that such an approach can provide a better understanding of the impact of pesticides on soil ecosystems. In particular, we examined the effects of fungicides on soil microbial activity (substrate-induced respiration and enzyme activities) and processes, which is essential for many biochemical and physical processes in soil. We also measured two processes that are mediated mainly by soil microorganisms (N mineralization and litter decomposition).

**MATERIALS AND METHODS**

**Design of soil microcosms**

Each microcosm consisted of a cylinder of 5.5 cm inside diameter \( \times \) 15 cm deep made of commercially-available high density polyethylene (HDPE) pipe (Fig. 3.1). Such a cylinder is suitable for this kind of study because it is impermeable, light-weight, rigid, and highly resistant to acids, bases, and biological degradation (Van Voris et al., 1985). Field-collected soil was roughly-sieved (6 mm) and packed gently into microcosms. The soil
Soil Microcosm

Fig. 3.1: Schematic diagram of the soil microcosm.
was a fine, mixed, mesic fraguidalf of the Canfield series from Wooster, Ohio. The soil is a
silt loam (13% sand, 73% silt, 14% clay) with 5% organic matter, 0.19% N, 10 cmolc kg\(^{-1}\)
cation exchange capacity, and a pH of 6.3. Different qualities of organic materials (ground
alfalfa leaves and ground wheat straw) were added to the soil at a ratio of approximately
50:1 (soil: amendment, dry wt basis) before adding any solutions. Fungicides (benomyl,
50% wettable powder and captan, 50% wettable powder) were applied to the soils by
spraying the fungicide solutions onto the soils (unamended, alfalfa-amended and straw-
amended) during thorough mixing. The final concentrations of fungicides were
approximately at the field rates (51 mg benomyl kg\(^{-1}\) and 125 mg captan kg\(^{-1}\) soil, active
ingredient). This was based on the assumption that surface-applied chemicals are
concentrated into the upper 2 cm of soil at a bulk density of 1.2 g cm\(^{-3}\). In addition to the
fungicide treatments, the solutions contained \(^{15}\)N labeled (NH\(_4\))\(_2\)SO\(_4\) (99.4 atom % \(^{15}\)N)
equal to 2.22 mg/kg soil (dry wt basis). This was used to determine the relative
contribution of the applied N vs. native soil N to plant uptake. Equal amounts of \(^{15}\)N-
labeled solution not containing the fungicide treatments were applied to additional batches
of soil to provide untreated control microcosms. All microcosms were kept in a
greenhouse under controlled temperature (28-30°C) and lighting cycles.

A small quantity (0.25 g) of chopped wheat straw contained within a small cylinder of
fiberglass screen material (1.6 x 1.8 mm mesh) was inserted into the top 5 cm soil of each
microcosm to measure the rates of organic matter decomposition. At the bottom of each
microcosm there was a layer of mixed-bed ion-exchange resins (7 g, Dowex MR-3 H\(^+\)
OH mixed bed resin) separated from the soil core by a thin layer of nylon stocking
material. This allowed free passage of soil leachate from the microcosms and served as a
partial barrier to prevent root growth out of the core bottom. Water was added two or
three times a week to maintain a soil moisture content between 30-40% (dry weight
basis).

Six winter wheat seeds (Triticum aestivum) were planted in the surface 0.5 cm of the
microcosm soil. After one week, germination success was recorded for each microcosm.
There were 4 replicate microcosms for each treatment, giving a total of 180 microcosms.
A subset of microcosms was sampled destructively after 3, 7, 15, 28, and 56 days. At the
end of each period, soil from each microcosm was removed, the litterbag and ion-
exchange resin bag were recovered. The soil was mixed gently, then subsampled to
determine soil enzyme activity (dehydrogenase, acid phosphatase and urease), substrate-
induced respiration, nitrogen nutrient status and rates of organic matter decomposition.
Wheat plants harvested from day 56 were separated by shoots and roots and analyzed for
biomass and tissue nutrient status.

Analyses

Measurement of soil microbial activity and microbial biomass

Microbial activity was assessed by measuring substrate-induced respiration (SIR),
soil dehydrogenase activity, acid phosphatase, and urease activity. SIR was determined as
the rate of CO$_2$ evolution during a 6 h incubation after adding 5 ml of glucose solution (32
mg ml$^{-1}$) to the soil. The evolved CO$_2$ was trapped in 0.02 N NaOH and precipitated with
excess 3 N BaCl$_2$, and then titrated with 0.02 N HCl with phenolphthalein (Anderson and
Domsch, 1973). Soil dehydrogenase activity (DHA), which is important in microbial
respiration and biomass, was determined by a modified method of Casida (1977) (Subler
et al., 1996). The enzyme-cleaved product (triphenylformazan) of the substrate of DHA,
2,3,5-triphenyltetrazolium chloride, was measured on a Lachat AE flow-injection
autoanalyzer at 480 nm. Soil urease activity (UREA), which is important in biochemical
nitrogen transformation in soils, was measured using a modified method of Tabatabai and
Bremner (1972). The NH$_4$-N released from hydrolysis of urea was determined in K$_2$SO$_4$
with the phenolate method using a Lachat AE flow-injection autoanalyzer with a 630 nm
filter. Acid phosphatase activity (PHOS), which is an important indicator of phosphorus
availability in soils, was assayed by the method of Tabatabai and Bremner (1969).
Microbial biomass N concentrations (BION) were determined using chloroform
fumigation-direct extraction method (Brookes et al., 1985).

Measurement of soil nitrogen pools

Extractable inorganic N concentrations (NH$_4$-N and NO$_3$-N) of the soil were
determined in 50 ml 0.5 M K$_2$SO$_4$ extracts (1:5 soil:extractant) using the phenate and
cadmium reduction/diazotization methods with a Lachat AE flow-injection autoanalyzer.
Dissolved organic N (DON) concentrations were calculated as the difference between the
extractable inorganic N concentrations and the NO₃-N concentration determined after alkaline persulfate digestion of the initial soil extracts (Cabrera and Beare, 1993). Net N mineralization rate was calculated as the difference in extractable inorganic N (NH₄-N + NO₃-N) between two dates. Nitrification rate was calculated as the difference in NO₃-N concentration between two dates.

Litter bag and ion-exchange resin bag

Litterbags were removed at the end of each sampling date, soil adhering to the wheat straw was carefully removed, and the straw was dried at 60°C for 48 h. Mass loss was assessed to estimate the rates of decomposition.

For quantification of NH₄-N and NO₃-N trapped by the ion exchange resin, the resin bags were washed with distilled water and dried at room temperature (Hubner et al., 1991). Then the adsorbed ions were then eluted with 50 ml 2 M KCl solution and determined on a Lachat AE flow-injection autoanalyzer.

Nitrogen uptake by plants

At the end of the study (56 days), above- and below-ground wheat biomass and tissue N concentration was determined. Shoots and roots were separated and the soil was removed from the roots by gentle shaking. The plant material was dried for 48 h at 60°C, or until constant weight, and shoot, root, and total biomass were recorded. Enrichment of ¹⁵N was determined by gas isotope ratio mass spectrometer (GIRMS) at Michigan State University.

Statistical analysis

SAS software (Statistical Analysis Software, Inc., 1990) was used for statistical analyses. Analysis of variance (ANOVA) was used to evaluate significant differences in individual response variables due to date for this microcosm experiment. Rate constants for decomposition and nutrient transformation processes were calculated. Orthogonal linear contrasts between each fungicide treatment and the untreated control for each dependent variable were obtained. Differences were considered significant at P < 0.05 unless otherwise indicated.
RESULTS

Effects of fungicides on soil microbial activity and biomass

Soil microbial activity (SIR, DHA and PHOS) and biomass (BION) except the urease activity was suppressed by all fungicide applications (Fig. 3.2-3.4). In the unamended soil, seven days after fungicide treatment, the SIR was significantly lower in soils treated with captan than in the untreated soils. This effect was significant even after 56 days. Soil DHA and PHOS had similar patterns to SIR but the significant effects were observed only for the first week for DHA and for two weeks for PHOS after the fungicide applications. In contrast, soil UREA was enhanced significantly by the fungicide treatments. Captan increased UREA on days 7 and 28 in the unamended and wheat straw-amended soils. A significant reduction of BION by captan was observed only on day 15 in the alfalfa-amended soil. It appeared that fungicide applications did not change microbial biomass dramatically but microbial activity was disturbed by the fungicides. Applications of benomyl resulted in similar effects to captan on soil microbial activity and biomass but had smaller influences on them.

Effects of fungicides on soil nitrogen pools

The different nitrogen pools in the soils were affected by the fungicide treatments dramatically as were the quality of the organic amendments (Fig. 3.4 and 3.5). NH₄-N concentrations in soils treated with captan were significantly higher than in the benomyl-treated and in the untreated soils, over all dates in the unamended and alfalfa-amended soils. This trend was consistent with increasing urease activity. The impact of captan on NO₃-N concentrations depended significantly on the different quality of the organic amendments. Captan increased NO₃-N to day 3 in the unamended soil and to day 28 in the
Figure 3.2: Substrate-induced respiration and soil dehydrogenase activity in microcosms treated with fungicides and amended with (A) unamended (control), (B) ground alfalfa leaves or (C) ground wheat straw, with benomyl (■), with captan (•) or without (○) fungicides (control). Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 3.3: Soil acid phosphatase activity and urease activity in microcosms treated with fungicides and amended with (A) unamended (control), (B) ground alfalfa leaves or (C) ground wheat straw, with benomyl (■ —■), with captan (● —●) or without (○ —○) fungicides (control). Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 3.4: Soil microbial biomass N (chloroform labile) and dissolved organic N concentrations in microcosms treated with fungicides and amended with (A) unamended (control), (B) ground alfalfa leaves or (C) ground wheat straw, with benomyl (■ ■), with captan (● ●) or without (○ ○) fungicides (control). Significant differences were indicated by + (0.05 < P < 0.10) and * (P < 0.05).
Figure 3.5: Extractable soil ammonium N and nitrate N concentrations in microcosms treated with fungicides and amended with (A) unamended (control), (B) ground alfalfa leaves or (C) ground wheat straw, with benomyl (■---■), with captan (●---●) or without (○---○) fungicides (control). Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
alfalfa-amended soil. On the other hand, DON concentrations were reduced significantly by captan in all soils especially during the first two weeks.

Benomyl had less effects on NH₄-N and DON than captan. NH₄-N concentrations increased significantly in response to benomyl in the beginning of the experiment in the unamended and alfalfa-amended soils. DON was also reduced by benomyl on day 7 in the unamended and alfalfa-amended soils and up to day 15 in the straw-amended soil. The significant effects of benomyl on NO₃-N were observed only in the first week in the straw-amended soil.

Effects of fungicides on soil processes

The soil processes measured in this study were rates of net N mineralization, nitrification, litter (wheat straw) decomposition and leaching of inorganic nitrogen concentrations from ion-exchange resin bags. The effects of fungicides on all of the above processes depended not only on the properties of fungicides but also the quality of the organic amendments. Captan enhanced the rates of net N mineralization significantly in the beginning then decreased them in the unamended and alfalfa-amended soils (Table 3.1). Initial rates of nitrification were increased by captan, but after 7 days captan significantly reduced the rates of nitrification and the effects lasted till the end of the experiment in the unamended and alfalfa-amended soils (Table 3.2). The litter decomposition followed similar trends to that of DHA. A greater percentage weight loss occurred with increasing period of time. The untreated controls had the greatest percentage weight loss from days 7 to 28. Captan inhibited litter decomposition significantly by 61% up to day 28 in the alfalfa-amended soil (Fig. 3.7). Increasing NH₄-N and NO₃-N in the ion-exchange bags were followed by initial increasing rates of net N mineralization and nitrification that were affected by captan, as well as by increasing NH₄-N and NO₃-N concentrations in the soils. The leaching rates for NH₄-N and NO₃-N were increased significantly by captan in all soils with exceptions of on day 28 in the amended soils (Fig. 3.6). Significant effects of benomyl on rates of net N mineralization were observed in the first week in the alfalfa-amended soil, but not thereafter (Table 3.1). The effects of benomyl on
Figure 3.6: Extractable ammonium N and nitrate N concentrations in ion-exchange resin bags in microcosms treated with fungicides and amended with (A) unamended (control), (B) ground alfalfa leaves or (C) ground wheat straw, with benomyl (■■■), with captan (●●●) or without (○○○) fungicides (control). Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 3.7: Percent mass loss (mean ± SE) of chopped wheat straw in litter bags in microcosms treated with fungicides and received ground alfalfa leaves. Significant differences were indicated by * (P ≤ 0.05).
rates of nitrification depended significantly on the quality of the organic materials. In the straw-amended soil, the initial rate of nitrification was increased significantly by benomyl, but after one week the rates were much lower than in the untreated controls (Table 3.2). Benomyl inhibited the rate of litter decomposition significantly by 81% to day 28 in the alfalfa-amended soil. On the other hand, benomyl and captan did not show any strong influences on rates of wheat straw decomposition in the unamended and straw-amended soils (Fig. 3.7). The leaching patterns of NH$_4$-N and NO$_3$-N found in benomyl-treated soils were different from those caused by captan. Benomyl had a significant effect on NH$_4$-N leaching only to days 15 and 56 in the unamended soil. The influence of benomyl on NO$_3$-N leaching depended on the quality of organic materials. Such significant effects were observed on days 3, 15 and 28 in the unamended soil, and days 15 and 56 in the straw-amended soil, respectively.

<table>
<thead>
<tr>
<th>Amendments</th>
<th>Fungicides</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>control</td>
<td>benomyl</td>
<td>captan</td>
</tr>
<tr>
<td>3</td>
<td></td>
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<td>0.10±0.022</td>
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<td>-0.57±0.173*</td>
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<tr>
<td>alfalfa-amended</td>
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<td>-0.045±0.009</td>
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<td>-0.01±0.033*</td>
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Note: significant differences $P \leq 0.05$, $0.05 < P \leq 0.10$ are indicated by * and +, respectively.

Table 3.1: Effects of benomyl and captan on net rates of N mineralization.
### Table 3.2: Effects of benomyl and captan on rates of net nitrification.

<table>
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<th>Amendments</th>
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<th>captan</th>
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<td>-0.142±0.069</td>
<td>-0.078±0.037</td>
</tr>
<tr>
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<td>-5.900±1.910</td>
<td>-6.350±0.586</td>
<td>-1.991±2.068</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.455±2.412</td>
<td>9.859±1.019*</td>
<td>-2.939±1.554*</td>
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<td></td>
<td>15</td>
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<td>-3.438±0.408+</td>
<td>0.851±0.443*</td>
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<td>28</td>
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<td>-0.448±0.359</td>
<td>1.368±0.376*</td>
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<tr>
<td></td>
<td>56</td>
<td>-0.254±0.058</td>
<td>-0.453±0.183</td>
<td>-1.169±0.168*</td>
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<td>wheat straw-amended</td>
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<td>-9.525±1.840</td>
<td>-1.263±2.562*</td>
<td>-4.383±0.539+</td>
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<td>-1.740±0.532</td>
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<td>-0.038±0.026</td>
<td>-0.036±0.020</td>
</tr>
</tbody>
</table>

Note: significant differences $P \leq 0.05$, $0.05 < P \leq 0.10$ are indicated by * and +, respectively.

### Effects of fungicides on plant growth

One week after the wheat seeds were sown into the microcosms, roughly one-third of the seeds had germinated. The rates of germination of wheat seeds were influenced significantly by the organic amendments and the fungicide applications. The alfalfa-amended soils treated with benomyl and captan had significantly lower germination than those of the untreated controls. On the other hand, both fungicides stimulated the rates of germination in the unamended and straw-amended soils, and benomyl significantly increased the germination rate by 62% in the unamended soil (Fig. 3.8). In general, microcosms with alfalfa-amended soil had a greater combined plant height than those with other soil amendments (Fig. 3.9). Related to the greater germination success, the microcosms with the greatest total seedling height after 56 days were those with untreated A soil. The plants in the alfalfa-amended soil treated with benomyl and captan had less
total height than those in the untreated control soil. The fungicide treatments resulted in significantly higher total wheat biomass (roots + shoots) compared to that of the untreated controls in the amended soils. Benomyl and captan significantly stimulated wheat growth by 60% in the alfalfa-amended soil and 248% in the straw-amended soil, respectively after 56 days (Fig. 3.10). The results for effects of fungicides on total shoot weight were similar to those for effects on total plant weight. There was a tendency for the fungicide treatments to increase the average shoot biomass, but there were no significant difference among them (P> 0.05) (data not shown). The mean shoot:root ratio of the wheat plants ranged between 1 and 2.5 (Fig. 3.11). The fungicide treatments significantly reduced this ratio in the W soil.

Effects of fungicides on plant uptake of nitrogen
Total nitrogen ($^{14}N$ and $^{15}N$) concentrations, $^{15}N$ enrichment, total and differential ($^{15}N$) nitrogen uptake were influenced significantly by the properties of fungicides and the organic amendments (Fig. 3.12, 3.13, 3.14 and 3.15). The total nitrogen uptake ($^{14}N$ and $^{15}N$) was enhanced significantly by captan in the amended soils after 56 days, although the total nitrogen concentrations in the wheat shoots were much less than the shoots in controls (Fig. 3.14 and 3.15). Benomyl did not affect the nitrogen uptake but reduced the amount of nitrogen in wheat shoots significantly after 56 days in the alfalfa-amended soil (Fig. 3.12 and 3.13).
Figure 3.8: Effects of the fungicides, benomyl and captan, on germination success (mean ± SE) of wheat seeds in microcosms that received different types of organic amendments. Significant differences were indicated by * (P ≤ 0.05).
Figure 3.9: Effects of the fungicides, benomyl and captan, on total shoot length (mean± SE) of wheat seeds in microcosms that received different types of organic amendments. Significant differences were indicated by * (P ≤ 0.05).
Figure 3.10: Effects of the fungicides, benomyl and captan, on total wheat biomass (shoots + roots) (mean ± SE) after 56 days in microcosms that received different types of organic amendments. Significant differences were indicated by * (P ≤ 0.05).
Figure 3.11: Effects of the fungicides, benomyl and captan, on shoot/root ratio of wheat plants (mean ± SE) after 56 days in microcosms that received different types of organic amendments. Significant differences were indicated by * (P ≤ 0.05).
Figure 3.12: Effects of the fungicides, benomyl and captan, on nitrogen concentrations (mean ± SE) in wheat shoots after 56 days in microcosms that received different types of organic amendments. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 3.13: Effects of the fungicides, benomyl and captan, on $^{15}$N enrichment of wheat shoots (mean ± SE) after 56 days in microcosms that received different types of organic amendments. Significant differences were indicated by * (P ≤ 0.05).
Figure 3:14: Effects of the fungicides, benomyl and captan, on nitrogen uptake by wheat shoots (mean ± SE) after 56 days in microcosms that received different types of organic amendments. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 3.15: Effects of the fungicides, benomyl and captan, on $^{15}$N uptake by wheat shoots (mean ± SE) after 56 days in microcosms that received different types of organic amendments. Significant differences were indicated by * (P ≤ 0.05).
The need for microcosm studies to determine the effects of fungicides on soil ecosystems originated from two important considerations: the fear of oversimplification and the problems associated with using xenobiotics or pollutants in field studies (Pritchard and Bourquin, 1984). Harmonized tests for assessing the potential impacts of a toxicant on an ecosystem parameters often involve single species or pure culture, and isolations of microorganisms from a complex natural mix. The justification for such tests is that the interactions among populations may be more sensitive to toxicants than that of individual species and that community studies may ultimately lead to a more quantitative estimation of ecosystem effects. The microcosm can be perceived as a potential laboratory tool that could help reduce this “fear of simplicity” by permitting the fate and effects studies to be made in much more ecologically complex laboratory systems. In addition, such a microcosm gives researchers the opportunity to alter environmental conditions, to simulate a large array of possible perturbations to the system. These options are usually not available in field studies. Moreover, the relatively small size of the microcosm used permits adequate replication, simplified dosing mechanics, control over inputs and outputs from the system, and adequate mixing, all factors are difficult to control in the field (Edwards et al., 1994; Pritchard and Bourquin, 1984).

Three types of microcosm devices can be distinguished (Heath, 1980; Ausmus et al., 1980): a closed type without free contact to air and moisture; a semi-open type with exposure only to air or moisture; and an open-type microcosm with free interaction with air and moisture. The microcosm unit used in this study can be qualified as open type systems. Microcosms differed from the field situation in terms of the influence of temperature and moisture dynamics, the influence of root presence, and the composition of the soil flora and fauna (Teuben and Verhoef, 1992). Many “ecosystem-level” behaviors are microbiologically-based processes, that reflect the integration of many internal processes and components, and their interactions within ecosystems (Schindler et al., 1980). These behaviors may include community respiration, nutrient cycling, oxidation-reduction gradients, organic matter transformation, and primary and secondary production. All of these properties cannot be inferred or predicted from measurements of isolated
components such as effects of chemicals on single species or mixed cultures in the laboratory (Weiss, 1971).

Several measurements have been considered to be good monitoring points to detect pollutant-induced changes in ecosystem behavior (Edwards et al., 1996; O’Neill et al., 1977; Pritchard and Bourquin, 1984; Van Voris et al., 1980). The leaching rates of nutrients from soil (O’Neill et al., 1977), \( \text{CO}_2 \) efflux from the respiration of soil microorganisms (Van Voris et al., 1980), effects on nitrogen cycle and redox potential (Eh) (Pritchard and Bourquin 1984). Disturbing the redox gradient could lead to significant changes in metabolic rates of carbon turnover and in mineralization by certain microbial communities. Redox potential could be measured by many ways including assays for dehydrogenase or electron-transport system (ETS) activity (Olanczuk-Neyman and Vosjan, 1977). However, it is difficult to assess the influence of pollutants on soil ecosystems without considering their potential interactive effects, because soil organisms including soil microbes and nutrient cycling processes are so intimately linked. Single-process tests have been used mainly to date in soil ecotoxicological research and environmental risk assessment using such tests could not provide data which can permit accurate prediction of potential environmental hazards of pollutants in field conditions (Edwards et al., 1996). An integrated soil microcosm of the type we described in this study could probably, provide the best laboratory information for overall predictions of potentially serious impacts of pollutants on soil ecosystems.

In present study, fungicide applications reduced microbial activity significantly (i.e. SIR, DHA and PHOS), but did not dramatically change microbial biomass N concentrations. A possible explanation was that the fungicides possibly shifted the balance of the soil microbial community from fungi to bacteria. The large flush of bacterial activity which followed the application of fungicides was probably responsible for many of the biochemical changes that occurred, particularly in relation to nitrogen mineralization (Wainwright and Pugh, 1974). In addition, the metabolic activity of a population relied upon the number of individuals as well as on the activity of each individuals; therefore, the effects of fungicide on a given activity were not necessarily correlated with a change in the
number of individuals that were responsible for the activity (Bollen, 1979). This hypothesis can be tested by separate measurements of fungal and bacterial activity and biomass. Hu et al. (1995) found that total soil N and microbial biomass N decreased significantly in the captan treatment and suggested an important role for fungi in the immobilization and retention of nitrogen in cultivated soils. It has been suggested that fungi could be responsible for the translocation and immobilization of N in natural ecosystems (Paustian, 1985) and non-tillage agroecosystems (Holland and Coleman, 1987; Beare et al., 1992). This probably became an alternative explanation for the relatively high urease activity and initial net N mineralization/nitrification rates, after fungicide treatments, because microorganisms needed a higher N requirement for repair and maintenance.

Captan has been generally regarded as having relatively limited non-target effects, compared with other fungicides which have been used to decrease populations of soil saprophytic fungi (Ingham et al., 1991). However, our results indicated that captan directly affected microbial activity and biomass in all small soil-plant microcosms. Benomyl showed similar patterns of effects on microbial activity and biomass as captan. Some researchers have claimed that benomyl and related fungicides are fungistatic in action and not fungicidal (Hofer et al., 1971; Raynal and Ferrari 1973). However, benomyl at 3 to 30 kg ha\(^{-1}\) had no effect on the rates of decomposition of organic matter and on size of bacterial populations of soils, but these treatments decreased nitrification after 4 weeks of incubation (Hofer et al., 1971). Wainwright and Pugh (1974) reported that benomyl increased ammonification, which resulted in an accumulation of ammonia in the soil, to a toxic level which could result in reductions in root length and plant height. However, to obtain conclusive data in view of their importance in determining the overall persistence of benomyl in the soil environment, tests on different soil properties and conditions should be processed (Vyas, 1988).

In the present study, the addition of wheat straw did not change soil microbial activity much compared to that in the unamended soil. Both unamended and straw-amended soils treated with either captan or benomyl had significantly restrained effects on microbial
activity and biomass N, although such inhibition was transient. Hart and Brookes (1996) examined two fungicides, epoxiconazole and triadimefon, and found that addition of wheat straw to the soil enhanced transient inhibition of ergosterol biosynthesis, and the inhibition was longer than in the unamended soil. On the other hand, the addition of ground alfalfa leaves had higher soil microbial activity and biomass. Zelles et al. (1985) also mentioned that addition of 0.5% alfalfa meal may destroy some of the smaller and finer differences between soils with applied fungicides and control soils, because the addition of alfalfa meal not only improved the conditions of the microorganisms but also a certain selection occurred. In addition, the improvement of soil by the addition of alfalfa meal promoted some reversibility of the effects caused by pesticides (Zelles et al., 1985).

An N priming effect, also referred to as the "added nitrogen interaction" (Molina et al., 1990) was observed in our study, although we did not directly measure gross N mineralization rates in the early investigation due to badly chosen methodologies. However, according to data on the nitrogen uptake by plants, captan in the amended soils increased total nitrogen uptake significantly which may suggest that captan application was able to speed up turnover rates of nitrogen, because the affected microorganisms had higher requirement of N. There were three possible reasons to explain the increased growth and N uptake responses observed after application of fungicides: (1) correction of wide soil-borne phytostasis, (2) decreased parasitic activity, or (3) increased mobilization and availability of N and/or release of nutrients by the plant probably because of inhibition of nitrification and/or denitrification. In contrast, some researchers reported that applications of fungicides reduced plant growth and nutrient uptake. For example, the growth of onions receiving fungicide treatments was reduced greatly by captan to 31% and by benomyl to 36% dry weight compared with plants in the untreated control soils (De Bartolde et al., 1978). Pentachlorophenol reduced the growth of birch and this could be partly a consequence of the effects of the fungicide on nutrient uptake processes, especially during the first growing period (Salminen and Haimi, 1996). Lowered nutrient uptake can be explained by direct growth inhibition of seedlings and/or disturbed root-mycorrhizae interaction. Reduced nutrient uptake by plants and reduced microbial
immobilization and/or nitrification were also indicated by the greater leaching of $\text{NH}_4^+-\text{N}$ from the most stressed soil than from the other soils (Salminen and Haimi, 1996).

CONCLUSIONS

The results all indicated that the quality of added organic matter strongly influenced the effects of the fungicides, benomyl and captan, on soil microbial activity and ecological processes. Captan suppressed N mineralization, apparently by reducing microbial activity, as indicated by short-term reductions in SIR and DHA. The two fungicides, captan and benomyl, differed in their effects on N mineralization, with captan having a differential effect, based on the quality of organic matter. Significant $\text{NH}_4^+-\text{N}$ accumulation over 56 days in the captan-treated soils compared to the other soils, probably resulted from lower rates of nitrification. The fungicide, captan, in comparison to benomyl, had a considerable impact on microbial activity and nitrogen transformations, an impact which was reflected in plant growth and N uptake. Results from our study suggest that, if a fungicide had to be chosen to control plant diseases, benomyl has less side-effects on soil microbial and biochemical processes, and the impact on soil environments will be much less compared to captan. However, the opposite conclusion can be drawn regarding the use of captan, it has to be used carefully in order to properly control plant pathogens and, at the same time, will not deleteriously damage well-balanced soil ecosystems and soil fertility.
CHAPTER 4

THE USE OF SOIL MICRO COSMS FOR ASSESSING THE EFFECTS OF FUNGICIDES IN SOIL ECOSYSTEMS: COMPARISONS OF TWO SOIL TYPES

SUMMARY

In this study, three broad-spectrum fungicides and two different types of soils were chosen to assess the impact of the fungicides on soil ecological processes and to establish the standardized evaluating approaches. The fungicides, benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate), captan (N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) and chlorothalonil (tetrachloroisophthalonitrile), are commonly used in current agricultural systems to control various plant diseases and to minimize disease incidence. A greenhouse microcosm approach was used to evaluate the effects of three fungicides on soil ecological processes in two soil types by measuring: (1) soil microbial activity and biomass, including soil substrate-induced respiration, soil enzymes (dehydrogenase and acid phosphatase activity) and microbial biomass nitrogen concentrations, (2) nitrogen transformations, including extractable inorganic nitrogen, dissolved organic nitrogen concentrations, net N mineralization and nitrification rates, (3) organic matter (litter) decomposition, and (4) plant growth. Each microcosm consisted of freshly-collected field soil, which was sieved, mixed and packed gently into a 5 cm x 15 cm high density polyethylene plastic cylinder. The effects of two concentrations of fungicides in two soils on soil processes were assessed at frequent intervals. The different
soil types and fungicide concentrations influenced the effects of the fungicides on soil microbial processes and N dynamics strongly. The sandy loam tended to have relatively higher microbial biomass and activity than the silt loam. Benomyl at both concentrations (51 and 510 mg kg⁻¹, a.i.) increased SIR, DHA, NH₄-N and DON, but reduced NO₃-N in both soils. The higher rate of benomyl (510 mg kg⁻¹) stimulated net N mineralization and nitrification rates initially but after 45 days, these rates were significantly lower than in the untreated controls in the silt loam. Captan significantly deteriorated soil microbial activity, especially at the higher rate (1250 mg kg⁻¹). Soil microbial biomass N as well as NH₄-N and DON concentrations were increased by the captan application, but amounts of NO₃-N were reduced. Like benomyl, captan increased rates of net N mineralization and nitrification initially but, after 45 days, both rates were reduced by the captan treatments. The effects of chlorothalonil on soil microbial activity depended on the nature of the soils. In the sandy loam, SIR, DHA and BION values were reduced significantly by the chlorothalonil treatments. Application of chlorothalonil had lower NH₄-N and higher NO₃-N and DON concentrations than the controls. The application of benomyl increased the leaching of NO₃-N from the soil systems, conversely, captan and chlorothalonil treatments reduced the leaching of NO₃-N. Fungicides did not influence the germination success of oat seeds after 7 days in the soil microcosms. Benomyl, at rate of 51 mg kg⁻¹ had relatively few significant effects on oat growth both in terms of plant height and weight in the silt loam. Neither captan nor chlorothalonil affected oat growth compared to the controls. Wheat straw decomposition rates were also inhibited by the fungicide application in the sandy loam. The three fungicides differed to some extent in their effects on soil processes and plant growth in the two soils; captan having a more profound and longer lasting influence than the other two fungicides. Taken together, we conclude that the microcosm technique can provide not only an accurate evaluation of the impact of fungicides on soil ecosystems, but also a better understanding of the interactions between fungicides applied and soil ecological processes and plant growth, as well as providing an idea how this interaction affects nutrient cycling and soil fertility in agricultural systems.
INTRODUCTION

Three commonly-used fungicides, benomyl, captan and chlorothalonil, are effective against a wide range of fungi and also have a potential for killing or affecting the function of a diverse range of soil organisms that contribute to soil biological processes and maintain soil structure and fertility (e.g. Edwards, 1988; Edwards and Bater, 1990; Vyas, 1988). Although a number of studies related to the effects of pesticides, mainly herbicides and insecticides, on soil ecosystems have been reported, very little research was done to examine the impact of fungicides on soil processes, based on interrelationships within microbial populations and community- or system-level interactions. In order to standardize methods for evaluating the impact of pesticides or soil pollutants on soil microorganisms, in recent years, some researchers have used microcosm techniques for ecotoxicological assays (Bogomolov et al., 1996; Driggan, 1977; Edwards et al., 1994; Gillett, 1989; Jackson et al., 1977; O'Neill et al., 1977; Parmelee et al., 1993; Sheppard 1997; Van Voris et al., 1985). Such microcosms have usually consisted of soil units containing multiple biotic species, and have advantages of being relatively simple, inexpensive and reproducible.

The literature on the effects of fungicides on soil organisms and soil processes is extremely diverse, ranging from the effects of fungicides on individual species of organisms, to those on overall populations of organisms and on individual biological systems using a great diversity of methods in both field and laboratory (Edwards and Bater, 1990). In addition, very little research has been done to examine the impact of fungicides on soil microbial activity and ecological processes (Tu, 1993) and little information is available to assess the effects of fungicides in microcosm studies (Takagi et al., 1991). Moreover, reports on the effects of fungicides on soil ecological processes and
nutrient cycles have been usually restricted to the application of single, or large doses in
the field or in the laboratory (Wainwright and Pugh 1973).

There are several reports that examined the effects of fungicides at various rates on
soil microorganisms and/or related processes. Captan has been applied at rates of 5 to
1000 mg kg\(^{-1}\) to observe its effects on soil microflora, microfauna and microbial activity,
either in the laboratory or in the field (Agnihotri 1971; Domsch 1959; Chinn 1973; Li and
Nelson 1985; Martinez-Toledo et al., 1997; Rapoport and Sanchez 1968; Tu 1981;
Wainwright and Pugh 1973). Extensive studies have been done to determine the effects of
benomyl on soil microorganisms and processes. The application rates of benomyl have
ranged widely from 1.2 to 1000 mg kg\(^{-1}\) (Li and Nelson, 1985; Newsham et al., 1994;
Newsham et al., 1995; Peeples 1974; Ramakrishna et al., 1979; Torstensson and Wessén
1984, van Faassen 1974) Some studies have been done to determine effects of
chlorothalonil, which is usually applied as a drench at rates of 15, 75, 150 and 1500 mg
kg\(^{-1}\), on cellulose decomposition (Katayama and Kuwatsuka 1991; Suyama et al., 1993).

Due to the strong influences of soil texture and properties on the behavior of
fungicides in soils, some fungicides have been tested in different types of soils. Martinez-
Toledo et al (1997) used four agricultural soils: two clay loam, silty loam and loam to
asses the effects of captan on soil microorganisms. Benlate (50% benomyl) was applied to
two humus-rich sandy soils (organic matter content, 8.9 and 9.7%, respectively) in the
laboratory (van Faassen 1974). Most of the studies comparing the effects in different soil
types have been done under field conditions and have focused primarily on benomyl (Li
and Nelson, 1985; Newsham et al., 1994; Newsham et al., 1995; Peeples 1974;
Torstensson and Wessén 1984). No studies have been done to look at the effects of
chlorothalonil in different soils.

In the present study, I adopted the methodology of Edwards et al. (1994) and applied
fungicides at two doses (a recommended field rate and 10 times of the rate) to two soils.
My main objective was to examine that whether the effects of three commonly used
fungicides on soil ecological processes and plant growth, differed between the chemicals
and two soils with distinct soil properties, summarize information obtained from this study and generalize how these impacts will affect the applications of fungicides to soil systems.

MATERIALS AND METHODS

Design of soil microcosms

Each microcosm consisted of a 5.5 cm (inside diameter) x 15 cm high cylinder made of commercially-available high density polyethylene (HDPE) tubing (Fig. 4.1). The tubing is suitable for this kind of study because it is impermeable, light-weight, rigid, and highly resistant to acids, bases, and biological degradation (Van Voris et al., 1985). Field-collected soils were sieved coarsely (6 mm) and packed gently into the microcosms. Two soil types were used in this study. One soil was obtained from Ohio State University Demonstration Farm, Reynoldsburg, Ohio. The soil is a fine, illitic, mesic aeric ochraqualf of the Centerburg series. The soil is a silt loam with 3.9% organic matter, 12 cmolc kg⁻¹ cation exchange capacity, and a pH of 7.2. Another soil was obtained from privately-owned woodland in Findlay, Ohio. The soil is a fine-loamy, mixed, mesic, typic hapludalfs of the Belmore series. The soil is a sandy loam with 4.7% organic matter, 14 cmolc kg⁻¹ cation exchange capacity, and a pH of 5.3. Fungicides (benomyl, 50% wettable powder; captan, 50% wettable powder; and chlorothalonil, 29.6% Daconil 2787®) were applied by spraying solutions onto the soils during thorough mixing. The final concentrations of fungicides were approximately the field rates (51 mg benomyl kg⁻¹, 125 mg captan kg⁻¹ soil and 54 mg chlorothalonil kg⁻¹, active ingredient (a.i.)), and 10 times of the rates (510 mg benomyl kg⁻¹, 1250 mg captan kg⁻¹ soil and 540 mg chlorothalonil kg⁻¹, a.i.). The calculations of field rates were based on the assumption that surface-applied fungicides are concentrated into the upper 2 cm of soil at a bulk density of 1.2 g cm⁻³. Untreated controls received only equal amount of deionized water. Five replicate microcosms were used for each treatment, with a total of 280 microcosms. The microcosms were kept in a greenhouse under controlled temperature (28-30°C) and lighting cycles.

A small quantity (0.25 g) of chopped wheat straw, contained within a small cylinder of fiberglass screen material (1.6 x 1.8 mm mesh) was inserted into the top 5 cm soil of
Soil Microcosm

Fig. 4.1: Schematic diagram of the soil microcosm.
each microcosm, to measure the rates of organic matter decomposition. A square of 2 x 2 cm anion-exchange membrane (type 204-UZRA Ionics Inc., Watertown, MA), was placed in the bottom 2.5 cm soil of each microcosm to \textit{in situ} measure soil NO\textsubscript{3}-N concentrations of the microcosms. The membrane squares were prepared by following the method of Subler et al. (1995). The microcosms stood on top of plastic petri dishes which allowed free passage of soil leachate from the microcosms and served as a partial barrier to prevent root growth out of the core bottom. Water was added two or three times a week to maintain a soil moisture content between 30-40% (dry weight basis).

Six oat seeds were sown in the surface 0.5 cm of the microcosm soil. After one week, germination success was recorded for each microcosm. A subset of microcosms was sampled destructively after 3, 10, 20, and 45 days. At the end of each period, soil from each microcosm was removed, and the litterbags and anion-exchange membrane were recovered. The soil was mixed gently, then subsampled to determine soil enzyme activity (dehydrogenase and acid phosphatase), substrate-induced respiration, nitrogen nutrient status. Small litter bags and anion-exchange membranes were recovered to determine organic matter decomposition and leaching rates of soil nitrate-N. Oat plants harvested from day 45 were measured for biomass and height.

\textbf{Analyses}

\textit{Measurement of soil microbial activity and microbial biomass}

Microbial activity was determined by measuring substrate-induced respiration (SIR), soil dehydrogenase, acid phosphatase, and urease activity. SIR was determined as the rate of CO\textsubscript{2} evolution, during a 6 h incubation after adding 5 ml of glucose solution (32 mg ml\textsuperscript{-1}) to the soil. The evolved CO\textsubscript{2} was trapped by 0.02 N NaOH, precipitated with excess 3 N BaCl\textsubscript{2}, and then titrated with 0.02 N HCl with phenolphthalein (Anderson and Domsch, 1973). Soil dehydrogenase activity (DHA), which is important in microbial respiration and biomass, was determined by a modified method of Casida (1977). The enzyme-cleaved product (triphenylformazan) of the substrate of DHA, 2,3,5-triphenyltetrazolium chloride (TTC), was measured on a Lachat AE flow-injection autoanalyzer at 480 nm. Acid phosphatase activity (PHOS), which is an important
indicator of phosphorus availability in soils, was assayed by the method of Tabatabai and Bremner (1969). Microbial biomass N concentrations (BION) were determined using chloroform fumigation-direct extraction method (Brookes et al., 1985).

**Measurement of soil nitrogen pools**

Extractable inorganic N concentrations (NH$_4$-N and NO$_3$-N) of the soil were determined in 50 ml 0.5 M K$_2$SO$_4$ extracts (1:5 soil:extractant) using the phenate and cadmium reduction/diazotization methods with a Lachat AE flow-injection autoanalyzer. Dissolved organic N (DON) concentrations were calculated as the difference between the extractable inorganic N concentrations and the NO$_3$-N concentration determined after alkaline persulfate digestion of the initial soil extracts (Cabrera and Beare, 1993). Net N mineralization rates were calculated as the differences in extractable inorganic N (NH$_4$-N + NO$_3$-N) between two dates. The nitrification rate was calculated as the difference in NO$_3$-N concentration between two dates.

**Litter bag and anion-exchange membrane**

Litterbags were removed at the end of each sampling date, soil adhering to the wheat straw carefully removed, and the straw dried at 60°C for 48 h. Mass loss was calculated to estimate the rates of decomposition.

For quantification of NO$_3$-N trapped by the anion exchange membrane, the membrane was washed with distilled water and dried at room temperature (Pare et al., 1995). The adsorbed ions were then eluted with 50 ml 2 M KCl solution and determined on a Lachat AE flow-injection autoanalyzer.

**Statistical analysis**

SAS software (Statistical Analysis Software, Inc., 1990) was used for statistical analyses. Analysis of variance (ANOVA) was used to evaluate significant differences in individual response variable due to date for this microcosm experiment. Rate constants for decomposition and nutrient transformation processes were calculated. Orthogonal linear contrasts between each fungicide treatment and the untreated control for each dependent variable were obtained. Differences were considered significant at $P < 0.05$ unless otherwise indicated.
RESULTS

The effects of three different fungicides on soil microbial activity and N dynamics depended significantly upon the applied fungicide concentrations and the properties of the soils used (Table 4.1). Significant interactions among soil types, fungicides and sampling time were found in SIR, DHA, NO$_3$-N, MINEN, NITR and AEM. A significant main effect of soil types was found in almost all measurements except NH$_4$-N and DON. Belmore sandy loam generally had relatively higher microbial activity and biomass than the silt loam.

Effects of benomyl on soil microbial activity and N dynamics

Both concentrations of benomyl increased peak SIR rates on day 10 in both soils, but had significant influence on SIR only in the silt loam (Fig. 4.2). Benomyl at 51 mg kg$^{-1}$ increased SIR, DHA and PHOS, but decreased BION concentrations in the sandy loam. A higher rate of benomyl stimulated SIR, DHA and BION, but had not effects on PHOS in two soils (Fig. 4.2-4.4). Benomyl at both concentrations increased NH$_4$-N and DON concentrations, but reduced NO$_3$-N concentrations in both soils. However, the higher rate of benomyl increased NO$_3$-N concentrations significantly on days 10 and 45 in the silt loam and increased DON in the sandy loam on day 45 (Fig. 4.6-4.8). At the beginning of the study, benomyl at both concentrations, stimulated rates of net N mineralization and nitrification significantly in the silt loam. However, these rates were reduced significantly by the higher rate of benomyl on day 20 in the silt loam. On the other hand, benomyl decreased the mineralization and nitrification significantly toward the end of the experiment in the sandy loam (Fig. 4.9 and 4.10).

Effects of captan on soil microbial activity and N dynamics

Captan significantly deteriorated soil microbial activity especially at the higher rate of captan (1250 mg kg$^{-1}$, a.i.). Soil DHA activity declined significantly at the higher rate of captan, in both soils, even after 45 days (Fig. 4.2). On the other hand, soil BION concentrations were increased by the captan treatments for the first three weeks, especially
Table 4.1: Significance of ANOVA model effects for soil microbial activity and nitrogen transformations measured 45 days after fungicide treatments in two soils.
in the sandy loam soil (Fig. 4.5). Different soil types also influenced the effects of captan on soil processes. Generally, the effects of captan at both concentrations were less in the sandy loam than in the silt loam. In the silt loam, captan at 1250 mg kg\(^{-1}\) significantly increased NH\(_4\)-N and DON concentrations, but decreased NO\(_3\)-N concentrations. The lower rate of captan (125 mg kg\(^{-1}\), a.i.) had similar effects on NH\(_4\)-N as the higher rate did. However the lower rate of captan tended to increase NO\(_3\)-N concentrations. In the sandy loam, NH\(_4\)-N and DON were promoted significantly by the captan treatment (Fig. 4.6-4.8). Net N mineralization and nitrification rates were enhanced by captan initially in both soils, but toward the end of the study, both rates were reduced by the fungicide treatments in the sandy loam (Fig. 4.9 and 4.10).

**Effects of chlorothalonil on soil microbial activity and N dynamics**

Chlorothalonil effects on soil microbial activity depended on the nature of the soils. In the silt loam, 54 mg kg\(^{-1}\) chlorothalonil increased SIR and DHA activity significantly, but had no apparent effects on PHOS (Fig. 4.2-4.4). However, BION concentrations were reduced significantly by the fungicide treatment (Fig. 4.5). In the sandy loam, SIR, DHA and BION were reduced by chlorothalonil treatment. The higher rate of chlorothalonil had more deleterious impact on soil microbial activity and biomass than the lower rate. Two concentrations of chlorothalonil acted differently on soil nitrogen transformations in both soils. The higher rate of chlorothalonil had greater NH\(_4\)-N and NO\(_3\)-N concentrations for the first 20 days and higher DON after 20 days than the lower rate. The application of chlorothalonil resulted in lower NH\(_4\)-N and higher NO\(_3\)-N and DON concentrations than the controls. Like captan, chlorothalonil also enhanced rates of N mineralization and nitrification, initially, but the effects lasted for less than 10 days. Chlorothalonil had more profound impacts on nitrogen transformations in the silt loam than in the sandy loam.

**Effects of fungicides on leaching of NO\(_3\)-N on anion-exchange membrane**

The amounts of NO\(_3\)-N absorbed by anion-exchange membrane (AEM) depended on the properties of soils (Fig. 4.11). The absorbed NO\(_3\)-N concentrations were
significantly higher in the silt loam than in the sandy loam. The application of benomyl increased the concentrations of NO₃-N absorbed on the AEM, which indicated more NO₃-N leached out of soil systems. The lower rate of benomyl had greater effects on leaching than the higher rate. The effect was also greater in the silt loam than in the sandy loam, with the exception of day 45, when 510 mg kg⁻¹ benomyl increased leaching of NO₃-N tremendously. The captan treatments reduced the amount of NO₃-N leached out of the soil systems, and especially, the higher rate of captan reduced the NO₃-N concentrations significantly in both soils. Similar to captan, chlorothalonil reduced the amounts of NO₃-N leached from the soil system. However, higher rate of chlorothalonil tended to increase the leaching than the lower rated did in both soils. In the sandy loam, 540 mg kg⁻¹ chlorothalonil had the highest leaching of NO₃-N among all fungicides except on day 45, where 510 mg kg⁻¹ of benomyl promoted the leaching of NO₃-N.

Effects of fungicides on litter decomposition

The nature of the two soils strongly influenced the effects of fungicides on the decomposition of chopped wheat straw (Fig. 4.12). One general trend was observed that the fungicides tended to enhance straw decomposition in the silt loam, but decreased the decomposition in the sandy loam. In the silt loam treated with 1250 mg kg⁻¹ captan, on the other hand, significantly decreased the straw decomposition after 20 days. In contrast, the sandy loam treated with the same rate of captan significantly increased straw decomposition during the same period.

Effects of fungicides on oat growth

One week after oat seeds were sown into the microcosms, roughly half of the seeds had germinated (Fig. 4.13). The rates of germination of oat seeds were linked to the concentrations of fungicides and the characteristics of soils. A comparison between the two soil types showed that in the sandy loam there was greater germination success, plant biomass and shoot height than in the silt loam. Benomyl, at the higher rate enhanced germination success of oat seeds significantly in the silt loam, but the oat biomass and
shoot height were the least in any fungicide treatment (Fig. 4.14 and 4.15). The lower rate of benomyl increased germination rates slightly and stimulated oat growth significantly in the silt loam. In the sandy loam, benomyl at both concentrations, resulted in slightly lower germination success rates and plant biomass, but had greater shoot height than in the control. Captan treatments did not cause any difference in germination success and plant biomass in the silt loam compared to the controls (Fig. 4.13 and 4.15). Conversely, the germination success and shoot heights in the sandy loam were less than the controls. The lower rate of captan had less influence on oat growth than the higher rate. Chlorothalonil acted differently in both soils. In the silt loam, the higher rate of chlorothalonil had the least germination rates, less shoot height and slightly greater plant biomass than the controls and the lower rate of chlorothalonil. In contrast, higher rate of chlorothalonil had higher germination rates but lesser plant biomass and shoot height than the controls and lower rate in the sandy loam (Fig. 4.14 and 4.15).
Figure 4.2: Substrate-induced respiration in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.3: Soil dehydrogenase activity in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.4: Soil acid phosphatase activity in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05< P < 0.10) and * (P ≤ 0.05).
Figure 4.5: Soil microbial biomass N (chloroform labile) in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.6: Extractable ammonium N concentrations in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.7: Extractable nitrate N concentrations in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.8: Dissolved organic N concentrations in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.9: Net N mineralization rates in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + ($0.05 < P < 0.10$) and * ($P \leq 0.05$).
Figure 4.10: Net nitrification rates in microcosms that received different fungicide treatments in two soils (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.11: Extractable nitrate N concentrations on anion-exchange membranes in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.12: Percentage mass loss (mean ± SE) of chopped wheat straw in litterbags after 45 days in microcosms that received different fungicide treatments in two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by * (P ≤ 0.05).
Figure 4.13: Effects of fungicides, benomyl, captan and chlorothalonil, on germination success (mean ± SE) of oat seeds in microcosms using two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
Figure 4.14: Effects of fungicides, benomyl, captan and chlorothalonil, on total plant height (mean ± SE) after 45 days in microcosms using two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P ≤ 0.10) and * (P ≤ 0.05).
Figure 4.15: Effects of fungicides, benomyl, captan and chlorothalonil, on total oat biomass (shoots + roots) (mean ± SE) after 45 days in microcosms using two soils. (A) silt loam, and (B) sandy loam. Significant differences were indicated by + (0.05 < P < 0.10) and * (P ≤ 0.05).
DISCUSSION

It is difficult to assess the influence of pollutants on soil ecosystems without considering their potential interactive effects, because soil organisms (including soil microbes) and nutrient cycling processes are so intimately linked. Single-process tests have been used to date in soil ecotoxicological research and environmental risk assessment could not provide a full picture of data which can accurately predict potential environmental hazards of pollutants under field conditions (Edwards et al., 1996). In order to obtain conclusive statements of the importance of soil type in assessing the overall impact and persistence of fungicides in the soil environment, tests on different soil properties and conditions should be determined (Vyas, 1988). An integrated soil microcosm, of the type we describe in this chapter, could provide the best information for overall predictions of potentially serious impacts of pollutants on soil ecosystems.

In the present study, the applications of benomyl increased SIR and DHA significantly in both soils. Some researchers claim that benomyl is fungistatic in action and not fungicidal (Hofer et al., 1971; Raynal and Ferrari, 1973). However, in the literature significant differences in DHA have been reported between soils (Central Point silt loam and Kubli loam) and sampling times (Li and Nelson 1985), but no significant effects of 20 or 200 ppm of benomyl in a humus-rich sandy soil (Van Faassen 1974). Benomyl did not alter the overall microbial populations of soils treated in the field at 2.2, 22.4 or 89.6 kg ha\(^{-1}\), and caused only slight increases in respiration rates of soils treated at 10 \(\mu\text{g g}^{-1}\) in the laboratory (Peeples 1974). These slight increases in respiration might result from the degradation of benomyl by soil microorganisms. After field applications of benomyl at 2.2, 22.4 kg ha\(^{-1}\) to a silt loam, fine sand soil and a sandy loam, no differences were observed in amounts of CO\(_2\) evolved from untreated turf soil (Peeples 1974). My results indicated that benomyl did not change the soil microbial biomass N concentrations significantly, but microbial activity was greatly influenced by benomyl in both soils.

Captan is a protectant-eradicant fungicide, which has been regarded generally as having limited non-target effects, relative to other fungicides used to reduce soil saprophytic fungi (i.e. Ingham et al., 1991). However, my results indicated that captan
retarded microbial activity drastically in the microcosms. Captan at a rate of 1250 mg kg\(^{-1}\) reduced SIR and DHA significantly but increased BION concentrations in both soils. This suppressive effect lasted until 45 days after the application of the fungicide to the sandy loam soil. An increase in BION might be due to the decomposition of killed fungi and other microorganisms (Agnihotri 1971) and an increase in bacterial populations (Wainwright and Pugh 1974). The metabolic activity of a microbial population relies upon the number of individuals as well as on the activity of each individual; therefore, the effects of fungicide on a given activity were not necessarily correlated with a change in the number of individuals that were responsible for the activity (Bollen, 1979). However, this hypothesis must be tested by separate measurements of fungal and bacterial activity and biomass. Hu et al. (1995) found that the total soil N and microbial biomass N decreased significantly in the captan treatment which suggested an important role for fungi in the immobilization and retention of nitrogen in cultivated soils. It has been suggested that fungi could be responsible for the translocation and immobilization of N in non-tillage agroecosystems (Holland and Coleman, 1987; Beare et al., 1992). Increases in crop yield due to increased nitrogen mineralization, following partial sterilization have been noted by several researchers (e.g. Widdowson and Penny, 1965; Salt, 1967; Cooke and Hull, 1972, Jenkinson et al., 1972).

Chlorothalonil had distinct patterns of effects on soil microbial activity and nitrogen dynamics compared to the other two fungicides. The effects of chlorothalonil depended on properties of the soils used. In the silt loam, SIR and DHA were increased by the fungicide treatment, however, in the sandy loam, both activities were inhibited. Takai et al. (1987) applied chlorothalonil (2.5 kg acre\(^{-1}\)) to a clay loamy soil of volcanic ash origin and found that the fungicide inhibited soil respiration and DHA, but increased total number of bacteria and gram-negative bacteria.

Anion-exchange membranes have been used to measure in situ soil N processes or N availability and used as alternatives to chemical extraction methods (Pare and Gregorich, 1995; Subler et al., 1995; Wander et al., 1995). Simulation modeling, soil column, and field studies have shown that soil texture and physical conditions, tillage methods,
agricultural chemical characteristics, and application practices are major factors in the retention, leaching and runoff of agricultural chemicals (Logan et al., 1987; Smith et al., 1991; Wauchope et al., 1992). Merwin et al (1996) reported that benomyl was prone to leaching or runoff from orchard soils under herbicide groundcover management systems. In my study, benomyl at both rates significantly increased NO₃-N absorbed on AEM which may imply that more NO₃-N ions tended to leach out of the soil systems.

There are three possible reasons to explain the increased growth and N uptake responses observed with application of some fungicides: (1) correction of wide soil-borne phytostasis, (2) decreased parasitic activity, or (3) increased mobilization and availability of N and/or release of nutrients by the plant probably because of inhibition of nitrification and/or denitrification. The captan treatment increased the survival odds of Douglas fir (Psudotsuga menziesii) (Colinas et al., 1994). Somda et al., (1991) reported that applications of benomyl and captan increased the total dry weight of tomato and total N uptake in the soils. In contrast, some researchers reported that applications of fungicides reduced plant growth and nutrient uptake. For example, the growth of onions receiving fungicide treatments, was reduced greatly by captan to 31% and by benomyl to 36% in dry weight than in control plants (De Bartolde et al., 1977, 1978). Wainwright and Pugh (1974) reported that benomyl increased ammonification which resulted in an accumulation of ammonia in the soil to a toxic level, which could have caused reductions in root length and plant height. Pentachlorophenol reduced birch growth and this could be part a consequence of the effects of the fungicide on nutrient uptake processes, especially during the first stage of the growth. The lowered nutrient uptake could be explained by direct growth inhibition of seedlings and/or a disturbed root-mycorrhizae interaction period (Salminen and Haimi, 1996). The reduced nutrient uptake by plants and reduced microbial immobilization and/or nitrification were also indicated by the greater leaching of NH₄-N from the most stressed soil than from the other soils (Salminen and Haimi, 1996).
CONCLUSIONS

The results indicated that both the doses of fungicides and the properties of soils influenced the effects of the fungicides, benomyl, captan and chlorothalonil, on soil microbial activity, nitrogen transformations and plant growth strongly. Benomyl enhanced soil microbial activity and the transformations of NH$_4$-N and DON which resulted in greater plant biomass and shoot heights in the silt loam. Captan suppressed N mineralization, apparently by decreasing microbial activity, as indicated by short-term reductions in SIR and DHA. Significant NH$_4$-N accumulation, over 45 days in the captan-treated soils compared to the other soils, probably resulted from lower rates of nitrification. The impact of chlorothalonil on soil processes and oat growth depended on the types of soils being used. Soil microbial activity/biomass and leaching of NO$_3$-N were both depressed by the chlorothalonil treatment. Oat growth was also retarded by chlorothalonil, although the effect was not significant statistically and amounts of soil NO$_3$-N and DON were increased by chlorothalonil. The fungicide, captan, compared with benomyl and chlorothalonil, has a considerable impact on microbial activity and nitrogen transformations, an impact which was reflected in plant growth. The results from this study suggest that, the best fungicide is to be chosen for controlling plant diseases, benomyl at regular field rates had less side-effects on soil microbial and biochemical processes, as well as less impact on soil environments than captan and plant growth was stimulated. However, caution has to be used because benomyl tends to increase the leaching of NO$_3$-N from the soil systems. An opposite conclusion can be drawn regarding the use of captan and chlorothalonil, because they have to be used carefully, to minimize disease incidence and at the same time should not damage well-balanced soil ecosystems and soil fertility. The conclusion from this study is that an integrated greenhouse microcosm approach, could be extended successfully to the investigation of the impact of pesticides or pollutants on soil ecological processes and plant growth.
CHAPTER 5

ASSESSING EFFECTS OF THREE FUNGICIDES: BENOMYL, CAPTAN AND CHLOROTHALONIL ON SOIL MICROBIAL PROCESSES AND OAT GROWTH IN FIELD

SUMMARY

In this study, the effects of benomyl, captan and chlorothalonil were examined in the field, to validate the predictive ability of the microcosm approach. Three broad-spectrum fungicides, benomyl, captan and chlorothalonil, which are commonly used in current agricultural systems, were chosen to assess the impact on soil ecological processes and oat growth. We measured: (1) soil microbial activity and biomass including soil substrate-induced respiration, soil enzymes (dehydrogenase and acid phosphatase activity) and microbial biomass nitrogen concentrations, (2) nitrogen transformations, including extractable inorganic nitrogen, dissolved organic nitrogen concentrations, net N mineralization and nitrification rates, (3) organic matter (litter) decomposition/breakdown and (4) plant growth. The effects of the fungicides at two rates (recommended field rate and 10 times of the rate) on soil processes were assessed at frequent intervals. The three fungicides did not affect soil microbial activity and biomass N concentrations significantly, compared to the untreated controls, except for an initial reduction of acid phosphatase activity was observed on day 3. Benomyl at both rates used did not influence soil NH₄-N, DON and in situ measurement of NO₃-N on anion-exchange membrane, however, benomyl increased initial soil extractable NO₃-N concentrations significantly. Like
benomyl, captan and chlorothalonil had similar patterns on the soil nitrogen transformations. The applications of fungicides enhanced the germination success and growth of oats with the exception of 380 mg kg\(^{-1}\) chlorothalonil. The results from the field study did not agree well with the results from the microcosm studies. The results from microcosm studies were more distinct and persistent for all of three fungicides tested.

**Key Words:** Fungicides, Benomyl, Captan, Chlorothalonil, Microbial activity, Soil enzymes, Nitrogen transformations, Litter decomposition, Anion-exchange membrane

**INTRODUCTION**

Pesticides, including fungicides, have the potential to kill or affect the function of a diverse range of soil organisms that contribute to the biological processes and maintain soil structure and fertility (e.g. Edwards, 1988; Edwards and Bater, 1990; Vyas, 1988). It is important to avoid contamination of soils by chemicals that have adverse effects on soil processes and thereby affect soil health and quality, for maintaining overall soil fertility. The fertility of soil, i.e. the capacity to produce acceptable crops, depends not only on its physical constitution and its nutrient content, but also on the intensity of the biological processes that take place within it. The increasing usage of pesticides, although intended to protect crops, may alter the biological equilibrium of the soil, by direct or indirect action, after a short, average, or long period of time, depending on whether the product acts quickly or persists longer in its initial state or metabolic forms (Simon-Sylvestre and Fournier, 1979). Therefore, there is a need to have a standardized methodology that will assess the potential hazard of any pesticides or soil pollutants in soil ecosystems (Edwards, 1988). In previous studies, we developed an innovative soil microcosm technique to examine the impact of three fungicides on soil ecological processes and plant growth. The simulation of ecosystem components in microcosms requires some verification or calibration, generally by making comparisons to field data (Pritchard and Bourquin, 1984; Van Voris et al., 1985).

Teuben and Verhoef (1992) reported that the reliability of results from open microcosm experiments need to be confirmed in the field, but this is rarely done. Field data
should infer that a degree of similarity (or dissimilarity) from data of laboratory and microcosms which have been established. Processes and responses in the field may differ considerably in magnitude, but should show the same trends as in microcosms (Pritchard and Bourquin, 1984; Van Voris et al., 1985). There have been some efforts to establish the parameters and responses of an ecosystem that are most suitable to compare differences between microcosms and the field and most of them have focused on aquatic microcosms (Pritchard and Bourquin, 1984). Ausmus et al (1978) used the evolution of CO₂ from soil, in comparing the litter decomposition patterns in a forest microcosm with field results under similar exposure to heavy metal. Van Voris et al (1985) found that a microcosm response to fly-ash treatment tracked the field results with respect to alfalfa productivity and trace element enrichment, but did not comply well with NO₃-N loss in leachate. There are many other potential field verification measures available. Almost any general parameter of microbial community structure and function, that is sensitive to various kinds of stresses or pollutants, is applicable as verifiable parameters between microcosms and the field. For example, adenylate energy charge (Chapman and Atkinson, 1977), a variety of enzymatic activities (Griffiths et al., 1982), and a whole range of heterotrophic activity measurements have been used (Pritchard and Bourquin, 1984).

The literature on the effects of fungicides on soil organisms and soil processes is extremely diverse, ranging from the effects of fungicides on individual species of organisms, to those on overall populations of organisms and an individual biological systems using a great diversity of methods in both field and laboratory (Edwards and Bater, 1990). The effects of benomyl and captan on soil organisms and microbial activities have been reviewed (e.g. Ingham, 1985; Schäffer 1993; Simon-Sylvestre and Fournier, 1979). More advanced studies have been done with captan, because it has been used widely for eliminating specific fungal groups in soil management systems (e.g. Beare et al., 1992; Beare et al., 1993; Ingham et al., 1986, Ingham et al., 1991; Hu et al., 1995). Little data in the literature is available to examine effects of chlorothalonil on soil microbial processes and its potential hazard to the environment. Furthermore, very little research has been done to examine the impact of fungicides on soil microbial activity as well as
ecological processes and their potential interactive effects (Edwards et al., 1996; Tu, 1993).

In the present study, we applied three fungicides at two doses (a recommended field rate and 10 times of the rate) to field plots. Our main objective was to verify that whether effects of three commonly used fungicides on soil ecosystems in the field are compared, within certain acceptable limits, with similar parameters measured in the previous microcosm studies. Moreover, we try to summarize information obtained from this study and generalize how applications of fungicides will cause possible hazard to soil systems.

MATERIALS AND METHODS

Design of field plots

Small quadrate plots (1 x 1 m) were established in Horticultural Research and Teaching Farm at the Ohio State University in Columbus, Ohio. This site has no pesticide application history for the past 10 years. The experimental design was a completely randomized block design, with four blocks, giving a total of 28 quadrat (control, 2 doses for each fungicide, and 4 replicates for each treatment). Space between quadrat was separated by mowed grass strips 1 m wide. Plants in the plots were thinned and they were weeded by hand as needed. The soil was a Crosby silt-loam Alfisol (fine, mixed, mesic Aeric Ochraqualf). A small quantity (0.25 g, dry wt.) of chopped wheat straw contained within a small cylinder of fiberglass screen material (1.6 x 1.8 mm mesh) was inserted into the top 5 cm of the soil to measure the rates of organic matter decomposition. A square of 2 x 2 cm anion-exchange membrane (AEM, type 204-UZRA, Ionics Inc., Watertown, MA), was tied with a fishing line looped through the membrane for easy retrieval and placed parallel to the soil surface, at a depth of 5 cm, by gently opening the soil with a garden shovel and firmly replacing the soil over the membrane to ensure uniform contact. The membrane squares were prepared by following the method of Subler et al. (1995). Water was added two or three times a week to maintain a soil moisture content between 30-40% (dry weight basis). Oat (Avena sativa) seeds were broadcast in the surface 0.5 cm
of the soil and covered with loose soil. After ten days, germination success was recorded for each plot.

**Weather conditions**

Average soil temperature from June to September 1996 was approximately 72.1 to 74.4 °F (Fig. 5.1) and the temperature maintained very consistent. The average rainfall ranges from 0.27 to 0.34 inch during the same period. After the application of the fungicides, the field received 5.76 inches of rainfall up to 10 days.

![Graph showing average soil temperature and rainfall from June to September 1996.](image)

Fig. 5.1: Average soil temperature and rainfall during the experimental period from June to September, 1996.
Fungicide treatments

Benomyl (50% wettable powder), captan (50% wettable powder), and chlorothalonil (29.6% Daconil®) were surface-applied evenly by watering cans to the treated quadrat, immediately following uniform cultivation of the surface soil (15 cm) with a rototiller and the planting of oat seeds at the same density as in the previous chapters. Final concentrations of fungicides used were approximately at the field rates (51 mg benomyl kg⁻¹, 125 mg captan kg⁻¹ soil and 38 mg chlorothalonil kg⁻¹, active ingredient (a.i.)), and 10 times of the rates (510 mg benomyl kg⁻¹, 1250 mg captan kg⁻¹ soil and 380 mg chlorothalonil kg⁻¹, a.i.). Calculations of field rates were based on the assumption that surface-applied fungicides are concentrated into the upper 2 cm of soil at a bulk density of 1.2 g cm⁻³. Untreated controls received only equal amounts of deionized water.

Soil sampling procedure

Three soil samples (15 cm depth, and 2 cm diameter) were taken from randomly selected points within each quadrat and bulked to give an composite sample for each quadrat 3, 10, 20, 45, and 90 days following the application of the fungicides. The soil was mixed gently, then subsampled to determine soil enzyme activity (dehydrogenase and acid phosphatase), substrate-induced respiration, nitrogen nutrient status. Litterbags and anion-exchange membrane were recovered to determine organic matter decomposition and in situ measurement of soil NO₃-N concentrations. Oat plants harvested from day 90 were weighted for biomass.

Analyses

Measurement of soil microbial activity and microbial biomass

Microbial activity was determined by measuring substrate-induced respiration (SIR), soil dehydrogenase and acid phosphatase activity. SIR was determined as the rate CO₂ evolution during a 6 h incubation, after adding 5 ml of glucose solution (32 mg ml⁻¹) to the soil. The evolved CO₂ was trapped by 0.02 N NaOH, precipitated with excess 3 N BaCl₂, and then titrated with 0.02 N HCl with phenolphthalein (Anderson and Domsch, 1973). Soil dehydrogenase activity (DHA), which is important in microbial respiration and
biomass, was determined by a modified method of Casida (1977). The enzyme-cleaved product (triphenylformazan) of the substrate of DHA, 2,3,5-triphenyltetrazolium chloride (TTC), was measured on a Lachat AE flow-injection autoanalyzer at 480 nm. Acid phosphatase activity (PHOS), which is an important indicator of phosphorus availability in soils, was assayed by the method of Tabatabai and Bremner (1969). Microbial biomass N concentrations (BION) were determined using chloroform fumigation-direct extraction method (Brookes et al., 1985).

Measurement of soil nitrogen pools

Extractable inorganic N concentrations (NH$_4$-N and NO$_3$-N) of the soil were determined in 50 ml 0.5 M K$_2$SO$_4$ extracts (1:5 soil:extractant) using the phenate and cadmium reduction/diazotization methods with a Lachat AE flow-injection autoanalyzer. Dissolved organic N (DON) concentrations were calculated as the difference between the extractable inorganic N concentrations and the NO$_3$-N concentration determined after alkaline persulfate digestion of the initial soil extracts (Cabrera and Beare, 1993). Net N mineralization rate (MINEN) was calculated as the difference in extractable inorganic N (NH$_4$-N + NO$_3$-N) between two dates. Nitrification rate (NITR) was calculated as the difference in NO$_3$-N concentration between two dates.

Litter bag and anion-exchange membrane

Litterbags were removed at the end of each sampling date, soil adhering to the wheat straw was carefully removed, and the straw was dried at 60°C for 48 h. Mass loss was calculated to estimate the rates of decomposition. For quantification of NO$_3$-N trapped by the anion exchange membrane, the membrane was washed with distilled water and dried at room temperature (Pare et al., 1995). Then the adsorbed ions were eluted with 50 ml 2 M KCl solution and determined on a Lachat AE flow-injection autoanalyzer.

Statistical analysis

SAS software (Statistical Analysis Software, Inc., 1990) was used for statistical analyses. Repeated measures analysis of variance (ANOVA) was used to evaluate significant differences of main effects in individual response variable for this study. If the repeated measures ANOVA was found to be significant, an univariate ANOVA was used
for subsequent analysis. An univariate ANOVA was used to determine significant differences in individual response variable due to dates in this study. Rate constants for decomposition and nutrient transformation processes were calculated. Differences were considered significant at $P < 0.05$ unless otherwise indicated.

**RESULTS**

A significant main effect of sampling time was found in all response variables. Significant interactions among block, time and concentrations of fungicides were found in SIR and $\text{NO}_3$-$\text{N}$ ($P \leq 0.10$), PHOS, MINEN and NITR ($P \leq 0.05$) (Table 5.1).

<table>
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<th>Variables</th>
<th>Effects</th>
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Note: Asteroid (*) indicates $P \leq 0.05$ and † indicates $0.05 < P \leq 0.10$.

Table 5.1. Significance levels of repeated measures ANOVA of individual response variables in field study.

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Effects of benomyl on soil microbial activity and N dynamics

Neither concentrations of benomyl affected soil microbial activity and biomass N concentrations compared to the untreated controls. However, benomyl at both rates significantly reduced soil acid phosphatase activity on day 3 (Fig. 5.3). Benomyl at 510 mg kg\(^{-1}\) generally had greater SIR and BION among all treatments (Fig. 5.2 and 5.3). Soil DHA was reduced by 51 mg kg\(^{-1}\) of benomyl but the effect was not significant (Fig. 5.2). Benomyl at both rates did not affect soil NH\(_4\)-N, DON and NO\(_3\)-N on AEM, but a great increase in NH\(_4\)-N was observed after 45 days (Fig. 5.4 and 5.5). In contrast, benomyl significantly increased extractable NO\(_3\)-N on day 10 and 20 followed by an increase in net nitrification rates (Fig. 5.4 and 5.6). Benomyl at both rates, significantly stimulated rates of net N mineralization and nitrification for the first 10 days, but not thereafter.

Effects of captan on soil microbial activity and N dynamics

Captan at both rates did not affect soil microbial activity significantly, except by causing decreases in soil PHOS on day 3 (Fig. 5.2 and 5.3). As a general trend captan at 1250 mg kg\(^{-1}\) decreased SIR, DHA and BION, compared to the untreated controls. Soils treated with captan had significantly increased extractable NO\(_3\)-N, but no effects on changes of NH\(_4\)-N concentrations (Fig. 5.4). Captan at 1250 mg kg\(^{-1}\) increased soil DON, with a significant effect on day 90, but NO\(_3\)-N on AEM was decreased by captan treatment (Fig. 5.5). The lower rate of captan had less dramatic effects on soil microbial activity and N transformations. Both rates of captan had different effects on soil net N mineralization and nitrification rates. Captan at a rate of 125 mg kg\(^{-1}\) increased initial MINEN and NITR significantly after 10 days. On the other hand, 1250 mg kg\(^{-1}\) captan reduced MINEN and NITR initially but significantly increased both rates by day 10 (Fig. 5.6).

Effects of chlorothalonil on soil microbial activity and N dynamics

Chlorothalonil did not affect soil microbial activity and biomass except that a significant reduction was found in PHOS on day 3 (Fig. 5.2 and 5.3). However, the lower
rate of chlorothalonil (38 mg kg\(^{-1}\)) tended to increase DHA and BION throughout the study. Moreover, the same rate of chlorothalonil increased extractable NO\(_3\)-N significantly after 10 days, which was followed by an increase in net nitrification over the same period (Fig. 5.4 and 5.6). In comparison with the higher rate of chlorothalonil (380 mg kg\(^{-1}\)), the lower rate had a more profound impact on nitrogen transformations.

**Effects of fungicides on oat growth**

Ten days after oat seeds were broadcast onto the soil, roughly 30% of the seeds had germinated. Although we did not see any significant effects of fungicides on oat growth (biomass and germination success rates), captan at the rate of 1250 mg kg\(^{-1}\) resulted in the highest germination percentage and the greatest oat biomass among the treatments (Fig. 5.7). Applications of all fungicides enhanced germination success and promoted oat growth, with the exception of 380 mg kg\(^{-1}\) chlorothalonil. A general trend was found for benomyl and captan that the higher the concentrations of the fungicides, the higher the germination success and biomass.
Figure 5.2: Effect of fungicides on (A) substrate-induced respiration and (B) soil dehydrogenase activity. Significant differences were indicated by * (P ≤ 0.05). CK for untreated control, BL for 51 mg kg⁻¹ benomyl, BH for 510 mg kg⁻¹ benomyl, CL for 125 mg kg⁻¹ captan, CH for 1250 mg kg⁻¹ captan, DL for 38 mg kg⁻¹ chlorothalonil, and DH for 380 mg kg⁻¹ chlorothalonil.
Figure 5.3: Effects of fungicides on (A) soil acid phosphatase activity and (B) microbial biomass N concentrations. Significant differences were indicated by * (P ≤ 0.05). CK for untreated control, BL for 51 mg kg\(^{-1}\) benomyl, BH for 510 mg kg\(^{-1}\) benomyl, CL for 125 mg kg\(^{-1}\) captan, CH for 1250 mg kg\(^{-1}\) captan, DL for 38 mg kg\(^{-1}\) chlorothalonil, and DH for 380 mg kg\(^{-1}\) chlorothalonil.
Figure 5.4: Effects of fungicides on (A) extractable NH₄-N and (B) extractable NO₃-N concentrations. Significant differences were indicated by * (P ≤ 0.05). CK for untreated control, BL for 51 mg kg⁻¹ benomyl, BH for 510 mg kg⁻¹ benomyl, CL for 125 mg kg⁻¹ captan, CH for 1250 mg kg⁻¹ captan, DL for 38 mg kg⁻¹ chlorothalonil, and DH for 380 mg kg⁻¹ chlorothalonil.
Figure 5.5: Effects of fungicides on (A) dissolved organic N concentrations and (B) NO$_3$-N concentrations on anion-exchange membranes. Significant differences were indicated by * (P ≤ 0.05). CK for untreated control, BL for 51 mg kg$^{-1}$ benomyl, BH for 510 mg kg$^{-1}$ benomyl, CL for 125 mg kg$^{-1}$ captan, CH for 1250 mg kg$^{-1}$ captan, DL for 38 mg kg$^{-1}$ chlorothalonil, and DH for 380 mg kg$^{-1}$ chlorothalonil.
Figure 5.6: Effects of fungicides on (A) net N mineralization rates and (B) net nitrification rates. Significant differences were indicated by * (P ≤ 0.05). CK for untreated control, BL for 51 mg kg⁻¹ benomyl, BH for 510 mg kg⁻¹ benomyl, CL for 125 mg kg⁻¹ captan, CH for 1250 mg kg⁻¹ captan, DL for 38 mg kg⁻¹ chlorothalonil, and DH for 380 mg kg⁻¹ chlorothalonil.
Figure 5.7: Effects of fungicides on (A) above ground oat biomass and (B) germination success (mean ± SE) of oat seeds. Significant differences were indicated by * (P ≤ 0.05). CK for untreated control, BL for 51 mg kg\(^{-1}\) benomyl, BH for 510 mg kg\(^{-1}\) benomyl, CL for 125 mg kg\(^{-1}\) captan, CH for 1250 mg kg\(^{-1}\) captan, DL for 38 mg kg\(^{-1}\) chlorothalonil, and DH for 380 mg kg\(^{-1}\) chlorothalonil.
DISCUSSION

The three fungicides, benomyl, captan and chlorothalonil used in our investigation caused only minor effects to soil microbial activities. Microbial populations always recovered quickly, so that side-effects had usually disappeared soon after the applications of the fungicides. All three fungicides had significant inhibitory effects on initial acid phosphatase activity on day 3 but not thereafter. Some researchers have claimed that benomyl is fungistatic, but not fungicidal, in action (Hofer et al., 1971; Raynal and Ferrari, 1973). Benomyl did not have any adverse influences on DHA (Li and Nelson, 1985) and gross microbial populations (van Faassen 1974), although a slight increase in respiration rates of soil treated with 10 µg g⁻¹ of benomyl occurred in the laboratory (Peeples 1974). Field applications of benomyl at 2.2, 22.4 kg ha⁻¹ to field sites did not show any differences in the evolution of CO₂ from soils (Peeples 1974). In the present study, the results indicated that benomyl did not change soil microbial activity and biomass N concentrations, which agrees with other results of field experiments (Peeples 1974; Smiley and Craven 1979; Wainwright and Pugh 1974). The literature reported ambiguous results of applying captan to field soils. Some workers considered captan has limited non-target effects relative to other fungicides (e.g. Ingham et al., 1991), but some found that captan decreased soil microbial biomass N and total soil N concentrations significantly (Hu et al., 1995). In the case of chlorothalonil, we did not find any major significant effects of this fungicide on soil microbial activity, but Takai et al. (1987) reported that chlorothalonil inhibited soil respiration and DHA but increased total numbers of bacteria.

In the present study, all three fungicides significantly stimulated extractable NO₃-N and DON concentrations. An increased flush of N increase in NO₃-N and DON was probably due to increases in the initial rates of net N mineralization and nitrification during the first 10 days. This increase was probably due to mineralization of dead fungi and other microorganisms, which provide readily available nutrients for the surviving population (Agnihotri 1971; Schuster and Schröder 1990; Torstensson and Wessén 1984). Ammonification was followed immediately by nitrification which resulted in significantly more NO₂-N and NO₃-N in the benomyl-treated soils (van Faassen 1974). Conversely, benomyl at 3 to 30 kg ha⁻¹ decreased nitrification after 4 weeks of incubation (Hofer et al.,
1971). Captan at the lower rates (0.5 to 10 kg ha\(^{-1}\)) either stimulated or had no effect on nitrification (Wainwright and Pugh 1973), but other workers have reported that captan at higher rates (62.5 to 250 ppm acre\(^{-1}\)) inhibited nitrification (Agnihotri 1971; Wainwright and Pugh 1974). The contradictory results in mineralization and nitrification confirm that this parameter has to be looked upon critically. Little information is available from the literature for chlorothalonil, nevertheless, this fungicide showed similar effects to benomyl and captan, on soil nitrogen transformations.

No significant effects of fungicide applications on oat growth were observed. However, a general trend was that applications of benomyl and captan, regardless of concentrations used, enhanced oat germination success and increased oat biomass. Increases in yield due to increased nitrogen mineralization following partial sterilization, have been noted by several researchers (e.g. Widdowson and Penny, 1965; Salt, 1967; Cooke and Hull, 1972, Jenkinson et al., 1972). The captan treatment increased the survival odds of Douglas fir (Colinas et al., 1994). Somda et al., (1991) found that applications of benomyl and captan increased the total dry weight of tomatoes and total N uptake in the soils. In contrast, some researchers reported that applications of fungicides reduced plant growth and nutrient uptake. For example, growth of onions receiving fungicide treatment was greatly reduced by captan to 31 % and by benomyl to 36% in dry weight than the control plants (De Bartolde et al., 1978). Wainwright and Pugh (1974) reported that benomyl increased ammonification, which resulted in accumulation of ammonia in the soil to a toxic level, which could result in reductions in root length and plant height. The effects of fungicides on plant growth, to some extent depend on the nature and doses of the fungicide, as well as characteristics of plants themselves.

CONCLUSIONS

Our results indicated that the application of fungicides at two different doses in the field did not have strong effects on soil microbial activity, nitrogen transformations and plant growth in the field. The results obtained from this study did not fully support our microcosm study data. Although the three fungicides did not influence soil microbial
activity significantly, a trend of succession of microbial populations in soils occurred. Furthermore, the partial stimulation of nitrogen transformations and enhancement of oat growth occurring, in our investigation, agreed with results of the microcosm studies. Benomyl enhanced soil microbial activity and NO$_3$-N as well as DON which resulted in greater plant biomass. Captan suppressed N mineralization initially apparently by reducing overall microbial activity, as indicated by short-term reductions in SIR and DHA. However, later increases in net N mineralization and nitrification resulted in significantly more NO$_3$-N and DON and greater plant growth. The impact of chlorothalonil on soil processes and oat growth was similar to that of benomyl and captan. The fungicide captan, at the higher rate compared with benomyl and chlorothalonil, caused a considerably dramatic impact on microbial activity and nitrogen transformations, an impact which is reflected in plant growth. Results from this study suggest that, all three fungicides at the recommended field application rates can be chosen to control plant diseases and should have few side-effects on soil ecological processes in agroecosystems.
GENERAL CONCLUSIONS

In my research, I was able to determine some distinct ecological side-effects of fungicides on soil microbial activity, nitrogen dynamics and plant growth. The results indicate that the overall impact of fungicides on soil systems depended on the nature and concentrations of the fungicides applied, different quality of the organic amendments, soil types and the environmental conditions. Fungicides inhibited as well as stimulated certain groups of microorganisms in the soil. All three fungicides had similar inhibitory effects on soil microbial activity and biomass, but the soil dehydrogenase activity was increased by the fungicide treatments in the laboratory batch incubations. Due to these changes, the influences of the fungicides on the different nitrogen pools depended on the structure and properties of the different fungicides. Each fungicide that was influenced by organic amendments, exhibited different effects on soil processes. In comparison to benomyl and chlorothalonil, captan appeared to have much greater effects on both soil microbial activity and nitrogen dynamics in the laboratory batch incubations.

In the microcosm studies, the results indicated that the doses of fungicides, different types of organic amendments and soil types influenced the overall effects of the fungicides on soil microbial activity, nitrogen transformations and plant growth strongly. Benomyl enhanced soil microbial activity and the transformations of NH$_4^+$-N and DON, which resulted in greater plant biomass and shoot heights in the silt loam. Benomyl, captan and chlorothalonil, differed in their effects on N mineralization, with captan having a different effect, based on the quality of organic amendments. Captan suppressed N mineralization initially, apparently by reducing the overall microbial activity, which was indicated by
short-term reductions in SIR and DHA. However, later increases in net N mineralization and nitrification resulted in significantly greater amounts of NO₃-N and DON and greater plant growth. Significant NH₄-N accumulation which occurred over 56 days in the captan-treated soils compared to the other soils, probably resulted from relatively lower rates of nitrification. The impact of chlorothalonil on soil processes and oat growth depended on the types of soils and organic amendments being used. Soil microbial activity and biomass were depressed by the chlorothalonil treatment. Oat growth was also retarded by chlorothalonil, although the effect was not significant statistically and amounts of soil NO₃-N and DON produced were increased by chlorothalonil. The fungicide, captan, compared with benomyl and chlorothalonil, had a considerable impact on microbial activity and nitrogen transformations; an impact which was reflected in plant growth and N uptake.

Results from the field experiment indicated that the application of the fungicides at recommended rates and ten times of the rates did not have strong effects on soil microbial activity, nitrogen transformations and plant growth. The results from this experiment did not support all of those from the microcosm research data. Although the three fungicides did not influence soil microbial activity significantly, a trend of succession of microbial populations in soils occurred. Furthermore, the partial stimulation of nitrogen transformations and enhancement of oat growth, occurring in our investigation, agreed with results of the microcosm studies. Additional research is needed to establish if the effects observed in this research can be integrated to provide an ecological risk assessment for these chemicals or other soil contaminants. Additional criteria that could be used include using intact soil cores instead of homogenized soils, testing more than two concentrations of the contaminant, including soil invertebrates into the system, examining the fate and transformations of the contaminant, applying tracer techniques (e.g. ¹³C, ¹⁴C, ¹⁵N, etc.), and exploring more appropriate methodologies, including molecular techniques to assess the overall impact of soil contaminants on terrestrial ecosystems. Nevertheless, my research provided strong evidence that agricultural practices (e.g. organic amendments) can influence the impact of fungicides on soil ecosystems significantly, when
the fungicides are applied to soil at the normal application rates. Furthermore, an integrated microcosm approach, could be used as a harmonized screening tool to investigate the impact of pesticides or pollutants on soil ecological processes and plant growth. Soil microcosms, when designed appropriately, appeared to be a powerful tool in predicting the environmental impact of chemicals on soil microbial processes as well as other soil organisms and processes. Among the three fungicides tested, when a fungicide is needed to control plant diseases, benomyl at the recommended field rates had least side-effects on soil microbial and biochemical processes, as well as impact on the soil environment, than captan and chlorothalonil, and in addition, plant growth was stimulated. These suggestions were based on greater soil microbial activity, higher nutrient (NO₃-N and DON) availability and accelerated plant growth.
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