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BY

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ABSTRACT

INTEGRATED DISEASE MANAGEMENT IN TOMATO PRODUCTION SYSTEMS

BY

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Early blight (EB), anthracnose (ANTH), and soil rot (SR), comprise a fungal disease pathosystem and bacterial-canker, -speck, and -spot, comprise a bacterial disease pathosystem that limit yield and fruit quality in midwest and Ontario tomato production systems. This thesis developed the premise that disease management strategies should reflect the divergent mechanisms by which the fungi and bacteria become epidemiological problems. The fungi reside in the agro-ecosystem, incite disease each year, are the target of regular fungicide applications and ultimately, are most effectively controlled through farm-level decisions and inputs. Three years of field research demonstrated EB, ANTH, and SR can be effectively managed through the integration of cultural practices such as conservation tillage and cover crops, that provided advantages consistent with a sustainable agriculture, and a disease forecasting model, TOM-CAST. Select forecast generated chlorothalonil spray schedules did not compromise yield of marketable fruit but required 45% to 80% fewer sprays compared to a standard weekly spray program. Reduced-sprays were most effective when combined with a zone tillage (ZT) system in 1990, a conventional tillage (CT) system in the absence of rotation in 1991, and ZT or rotation in 1992.

The bacterial pathosystem is likely to be controlled through genetic resistance and prevention (i.e. disease free seed and transplants). Effective control is therefore dependent on knowledge of the genetic diversity of each pathogen. The genetic

diversity of a world-wide collection of over 80 isolates of the spot organism, *Xanthomonas campestris* pv. *vesicatoria* (Xcv) was assessed using repetitive DNA sequences and the polymerase chain reaction (PCR). Based on fingerprint patterns generated from total genomic DNA, the protocol known as rep-PCR, delineated 4 diverse evolutionary lineages classified as Xcv. One lineage, designated as Group B, was newly described as an important component of the spot disease complex in the midwest.

Integrated disease management in tomato production systems is possible through the combination of farm level activity, such as conservation tillage and rotation, and supra-farm-level activity, such as biotechnology based protocols.

Additional Key Words: sustainable agriculture, reduced pesticides, reduced tillage, REP-, ERIC, BOX-PCR, genetic fingerprinting, bacteria

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"When a farmer plows for
planting, does he plow continually?
Does he keep on breaking
up and harrowing the soil?
When he has leveled the
surface,
does he not sow caraway
and scatter cummin?
Does he not plant wheat in its place,
barley in its plot,
and spelt in its field?
His God instructs him
and teaches him the right
way.
All this comes from the
Lord Almighty,
wonderful in counsel and
magnificent in wisdom."

Isaiah son of Amoz, 725 B.C.

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CHAPTER I

GENERAL INTRODUCTION AND LITERATURE REVIEW

A. INTRODUCTION

Over 25,000 ha of tomato are grown in Michigan, Ohio, Indiana and Ontario (the northcentral production region) with an estimated farm gate value of 145 M US dollars (OMAF 1990; USDA 1990). Current yields of up to 70 T ha^{-1} are not uncommon in the processing tomato industry, as compared to 5 to 8 T ha^{-1} recorded by Brown in 1929 (Brown 1929). Crop productivity has been enhanced through superior tomato cultivars, specialization and mechanization, intensive tillage of soils combined with high fertilizer inputs, and significant chemical-dependent advances in weed, insect and disease control. However, the industry currently faces numerous challenges. Synthetic chemicals are facing an unprecedented challenge including consumer, regulatory agency, environmental and grower safety concerns. Likewise, intensive tillage of the land and energy-intensive inputs are seen as counter-productive to long-term sustainability. A movement toward a sustainable agriculture impinges on current chemical dependent disease control and agronomic practices even though there is a lack of adequate alternatives such as cultural, genetic or biological disease control strategies and reduced tillage systems.

Fungal and bacterial diseases limit yield and product quality yearly and are favored by the climate of the northcentral region. This thesis focuses on integrated disease management of field-tomato diseases within a context of current tomato

production systems. Field research was conducted in southwest Michigan where many growers farm on light sandy soils and follow an intense biennial cropping sequence of tomato and cucumber crops. With regard to disease management, this thesis takes two approaches. The first approach may be considered "grower dependent" integrated management. Management of diseases within this context occurs primarily at the individual farm enterprise level. Each grower manages knowledge and material inputs for disease control within their site specific production system. The second approach may be considered "industry dependent" integrated management where "industry" refers to the tomato industry as a whole including private and public institutions. At this level, inputs are first of all knowledge based and are integrated for disease management before the seed arrives at the farm gate.

The objective of this study was to: 1) minimize the number of fungicide applications required for control of early blight, anthracnose and soil rot of tomato through the use of tillage, a green manure crop, and weather timed fungicide sprays within a biennial (tomato/cucumber) conservation tillage production system, without compromising fruit quality and yield, and,

2) to assess the genetic diversity of bacterial pathogens of tomato, using the bacterial spot organism as a model, and ascertain how genetic diversity impacts industry dependent disease management strategies.

The literature review will highlight components included in the tomato production system within a broader context of sustainable agriculture.

B. LITERATURE REVIEW:INTEGRATED DISEASE MANAGEMENT AND SUSTAINABLE AGRICULTURE

1. INTEGRATED DISEASE MANAGEMENT

Integrated disease management is a form of the generic integrated pest management (IPM) or integrated pest control (IPC) and is defined as "a pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible manner as possible and maintains the pest populations at levels below those causing economic injury" (FAO/UNEP 1984). The concept of IPM has progressively evolved since Stern et al. (1959) first introduced it. Originally, IPM grew in response to increasing crop damage and pest resistance to chemical based controls (Cooley 1993). Today, the concept has evolved to include broad goals including environmental and social issues, and has become a mainstay in the sustainable agriculture movement (Poincelot 1986).

2. SUSTAINABLE AGRICULTURE

Sustainable agriculture is a philosophy of agriculture reflecting human goals and embraces a broad range of definitions found throughout the literature. Most definitions of sustainability encompass rural community and farm family socio-economic aspects, preservation of non-renewable agro-ecosystem resources (water, soil and biodiversity), the need for an adequate food and fiber supply to meet the needs of a growing population, and optimization of long term farm enterprise profitability and productivity, presumably over an indefinite time frame. Allen et al. (1991) perceived

that sustainable agriculture has a focus on the entire global food and agriculture system that does not just include environmental and economic viability, but also social justice for all sectors of society.

The 1990 United States farm bill has less of a global perspective and defined sustainable agriculture as "an integrated system of plant and animal production practices having site specific application that will, over the long term:satisfy human food and fiber needs; enhance environmental quality and the natural resource base upon which the agriculture economy depends; make the most efficient use of non-renewable resources and integrate, where appropriate, natural biological cycles and controls; sustain the economic vitality of farm operations; and enhance the quality of life for farmers and society as a whole" (Bird 1993). Definitions lose a sociological tone and progressively become more practical oriented when applied to the actual process of farming. For example, Francis et al. (1987) define sustainable agriculture as a "result of a management system which helps the producer to choose the hybrids and/or varieties, soil fertility packages including rotations, pest management approaches, and cropping sequences to reduce costs of purchased inputs, minimizing the impact of the system on the immediate off-farm environment, and provide for a sustained level of production and profit from farming".

At the farm enterprise and field level, Fretz et al. (1993) suggest sustainable agriculture requires more of a "process oriented" and "problem-solving" mentality as compared to a "product oriented" mentality. Information and management are said to substitute for non-renewable, energy-intensive inputs. At the farm level, many published works envision improved crop rotations, use of legumes in cover crop sequences, improved nutrient cycling, and a livestock component. Wien (1990) notes

that farm-level sustainability is not stepping back into the past but requires "a much more sophisticated and detailed understanding of the agriculture ecology ...[including]...pest and predator populations and their dynamics...". The latter definition of Francis et al. and expanded on by Fretz et al. and Wien provided a functional framework for this thesis.

3. SELECTED COMPONENTS OF FARM-LEVEL SUSTAINABLE PRACTICES IN VEGETABLE PRODUCTION SYSTEMS

Sustainable farming practices have not been adopted with equal success in vegetable production systems as compared to field crop or mixed farming operations (Kelly 1990). For example, systems that include tomato production, especially fresh market tomato production systems, are specialized and do not have crop diversity or land resources to include long crop rotations, legume based crops or reduced tillage practices. Considerable research is required to make vegetable production systems more sustainable and less input intense. For more than a decade, researchers at Michigan State University have examined the potential of a more sustainable tomato production system (Barnes and Putnam 1983; Drost 1983; Grajauskis 1990; Jardine et al. 1988; Price and Baughn 1987; H. Price and C.T. Stephens unpublished; Putnam 1990). Key components include conservation tillage practices, use of cover and green manure crops, and reduced pesticide input.

Conservation tillage systems and use of cover crops are integral components of sustainable production. The practices have many advantages but also some draw backs (Coolman and Hoyt 1993; Kenimer et al. 1986; Morse 1993; Phillips et al. 1980; Sarrantonio 1992; Shennan 1992; Spieser 1983). Advantages include dramatic

reductions in wind and water soil erosion; enhanced water use efficiency; improved soil moisture content; enhanced soil productivity; decreased dependence on non-renewable energy; increased nutrient recycling; elevated microbial activity; decreased pesticide losses and improved timing of harvesting and planting. Disadvantages include increased soil compaction problems; altered weed and disease populations that may require additional pesticide inputs; reduced germination or crop stands, and decreased soil temperatures. Success with specific tillage practices and cover crops is influenced by soil type, cropping system, environment and choice of cover crop among other components (Benoit and Lindstrom 1987; Sarrantonio 1992).

a. CONSERVATION TILLAGE

Conservation tillage is defined as "any tillage and planting system that retains at least 30% residue cover on the soil surface after planting (Hiemstra and Bauder 1984). Variations, as defined by the Soil Science Society of America (1978), include: no-tillage (NT) - "a crop production system whereby a crop is planted directly into a seedbed not tilled since harvest of the previous crop"; and minimum tillage - "the minimum soil manipulation necessary for crop production or meeting tillage requirements under the existing soil and climatic conditions". Conventional tillage (CT) reflects the normal procedures for crop production in a given region but usually consists of a primary deep tillage operation (moldboard plow) followed by one or more passes of secondary tillage (Soil Science Society of America 1978; Phillips et al. 1980).

Conservation tillage practices are common and successful in many field crop production systems (Phillips et al. 1980) but provide variable results in tomato

production systems. Beste (1976) observed yields of tomatoes direct seeded into a rye stubble mulch (NT) were equal to yields in a CT system. Knavel et al. (1977) transplanted tomatoes for two years into a sod cover and a third year into a wheat mulch. The sod and wheat NT systems did not affect transplant survival but decreased and had no affect on yield, respectively, as compared to CT. Shelby et al. (1988) transplanted tomatoes into a desiccated wheat cover and obtained nearly twice the marketable yields as compared to CT. In a second year, NT and CT yields were similar.

Doss et al. (1981) observed marketable yield of fresh market staked tomatoes tended to decrease as the amount of tillage decreased. Treatments included in row chiseling combined with direct field setting of tomato transplants into a rye mulch (NT), strips of incorporated rye mulch, or CT plots. They noted early plant growth decreased in rye plots. The impact of NT using a rye cover crop was also noted by Price and Baughn (1987) and Grajauskis (1990). Early plant growth and fresh market tomato yields were consistently depressed in their NT system.

Associated yield decline with rye mulch cover has led to modified minimum tillage systems such as strip tillage (ST). McKeown et al. (1988) tilled strips into killed rye or oat cover crops approximately 3 weeks prior to transplanting tomatoes. Yields in all plots were similar for 2 years but depressed in rye ST plots a third year. Grajauskis (1990) employed a modified ST system. Rye strips were incorporated in early spring and the remainder of the rye was desiccated at a later date prior to planting. Soil was fractured directly below where tomato plants were to be established (zone tillage ZT). The combination of ST and ZT enhanced marketable yields as compared to NT or CT and enhanced plant productivity was associated with a more

extensive root system in ZT plots (Grajauskis 1990). Hedgewood et al. (1978) also noted subsoiling benefited tomato productivity. Sumner et al. (1981) have associated subsoiling with enhanced root penetration and greater efficiency of nutrient uptake as opposed to impact on root disease incidence.

Weed control is a recurring problem with reduced tillage in vegetable production systems (Knavel et al. 1977; Putnam 1990). In transplant tomato production, pre-plant incorporation of trifluralin provides early season control of germinating weed seeds but trifluralin cannot be applied in reduced tillage systems. However, recent advances in post emergent selective herbicides allow for adequate weed control in reduced tillage systems (Putnam 1990; Wallace and Bellinder 1992). Shelby et al. (1988) demonstrated the potential of metribuzin and sethoxydim for post emergent control of broad leaf weeds and grasses, respectively, in tomatoes.

Most conservation tillage experiments in vegetable production systems do not include a plant pathology component (Sumner et al. 1986). Jardine et al. (1988) observed a higher incidence of bacterial speck on tomato plants in NT compared to CT plots infested with infected debris the previous fall. McKeown et al. (1988) observed bacterial speck incidence was higher in ST in 1 out of 3 years and plant parasitic nematode populations were stimulated by ST. Sumner et al. (1981 & 1986) have observed that conservation tillage practices increase, decrease or have no effect on plant diseases. They summarize that tillage impacts *Rhizoctonia* populations but generally not *Fusarium, Pythium* or nematode populations. In general, they observed root diseases are affected more by the previous crop than by tillage practices. Few studies have examined the impact tillage has on the incidence of foliar diseases of vegetables. Sumner et al. (1986) surmise that burial of crop debris may reduce initial

inoculum but have no effect on secondary spread of foliar pathogens, except indirectly through altered host productivity and reaction to infection.

b. COVER CROPS

Cover crops have been used in crop production systems for thousands of years to enhance soil fertility and physical properties (Sarratonio 1992). Numerous cover crops have proven successful in vegetable production systems (Shennan 1992) and choice of cover crop is dependent on the grower's goals. Legume crops provide nitrogen and are preferred for long-term sustainability (Frye and Blevins 1989). However, legume crops require relatively long growth periods to achieve benefits. This often precludes their use in vegetable production systems. Rapid growing cover crops have the advantage of quickly tying up soil nutrients and are most suitable to fill windows of opportunity in vegetable production systems (Sarrantonio 1992). Rye (*Secale cereale* L.) is often preferred. Fall sown rye is winter hardy and rapidly acquires biomass in the early spring as compared to oats and wheat.

Rye mulch can act as a smother crop to reduce weed populations. Rye residue also releases allelopathic substances that can be exploited for early-season weed control (Barnes and Putnam 1987; Putnam 1990) although allelopathy may also be responsible for decreased tomato productivity (Grajauskis 1990). Putnam (1990) summarizes that rye on the soil surface releases chemicals that are highly inhibitory to dicotyledonous weed seedlings and offers variable control of grassy weeds. Weed management systems have been effective in tomatoes using rye residue for early season control and "rescue" treatments of metribuzin or sethoxydim applied as-needed for control of later-season weeds (Putnam 1990; Wallace and Bellinder 1992). Putnam (1990) notes reduced rates of post- emergent herbicides can be used with optimum

timing. Such a system requires close monitoring of weed populations.

Cover crops have also been exploited to reduce insect damage (Bugg 1992) and wind damage to plants (Spieser 1983; Beste 1973). High winds are associated with decreased yield potential in tomatoes (Armburst et al. 1969) and sand blasting injury which has been associated with increased incidence of disease in tomato fields (Vakili 1967; Rotem 1965).

Limited research has been published concerning the impact of traditional cover crops on disease incidence in vegetable cropping systems. A rye cover crop reduced early-season severity of corky root rot of lettuce but this may have been associated with altered soil physical properties (van Bruggen et al. 1990). In contrast, plant members of the *Cruciferae* family have proven activity against plant pathogens surviving in soils (Mojtahedi et al. 1993; Muehlchen et al. 1990). Sulphur containing glucosinolates present in tissues of *Cruciferae* plants hydrolyze enzymatically to form a number of volatile compounds including isothiocyanate, chemical structures similar to those used in commercial fumigants. The sulphur containing compounds are speculated to diffuse in soil and act as fumigants against soilborne pathogens (Lewis and Papavizas 1970).

c. REDUCED PESTICIDES

Public concern, farm profitability, cancellation of registered fungicides and lack of new chemistry, restrictive legislation, and pest resistance (Merwin and Pritz 1993; Stephens 1990) is forcing vegetable growers to reduce pesticide inputs.

Currently, fungicides are applied on nearly 100% of tomato hectarage in the northcentral production region (Precheur et al. 1992). Most growers follow the

standard recommendation to initiate sprays when fruit first set and to apply subsequent sprays every 7 to 14 days even if the risk of disease is zero. Up to 12 or more applications are required each season. However, data over the last several years suggest a reduced number of precise timed fungicide applications can be used to control fruit rots in tomato, an approach more compatible with goals of a sustainable agriculture.

Disease forecasting systems are designed to determine the need for the initiation and/or timing of subsequent pesticide applications for the purpose of reducing disease incidence and efficient use of resources. Early blight of tomato is a "classic" example of research efforts to design efficient spray programs. Martin (1920) noted early season sprays (in this case copper based "fungicides") could be eliminated without compromising disease control. Horsfall and Heuberger (1942) likewise concluded, for the northeastern production region, "July 10 is early enough [to initiate a spray program] in most years". During the late 1970's, control was evaluated using the "Massive Dosage Technique" (Pitblado 1992; Stevenson 1977). The primary goal of massive dosage, prior to pesticide issues moving to the forefront of the public conscience, was to reduce time devoted to fungicide applications. Fungicides were applied at 2 to 3 fold rates on a reduced schedule, compared to recommended rates every 7 to 10 days. Limited success was achieved and multiple applications continued to prove more successful (Stevenson 1977).

Waggoner and Horsfall (1969), in their classic publication "EPIDEM", designed a Fortran based computer simulation of early blight disease progress. The program was based on considerable original work, previously published work and over a decade of notes on monitored early blight epidemics. EPIDEM, and work by others, was

synthesized to formulate FAST, a Forecast system for *Alternaria Solani* on Tomato (Madden et al. 1978). FAST incorporated two empirical models utilizing daily maximum and minimum air temperature, hours of leaf wetness, maximum and minimum temperature during the wetness period, and hours of relative humidity greater than 90% and rainfall. FAST effectively controlled early blight with fewer spray applications as compared to weekly applications (Madden et al. 1978; Pennypacker et al. 1983).

The multiple environmental parameters required by FAST and the cumbersome equipment (Taylor dew meter and hygrothermograph) required to monitor the weather patterns, limited the application and general use of FAST. From 1983 to 1988, Dr. Ron Pitblado at the Ridgetown College of Agricultural Technology, Ridgetown, Ontario, evaluated and modified FAST to be less complex and, effective for control of early blight, anthracnose and *Septoria* leaf spot (Pitblado 1988; 1992). Pitblado simply used a table, devised by Madden et al. (1978), to calculate daily disease severity values (DSVs) based on the average temperature during hours when foliage are wet. The modified program was called Tom-Cast for **TOM**ato disease fore**CAST**er. According to the Tom-Cast model, the first spray is applied on July 11 or earlier if 28 days have lapsed since transplanting and if the DSV has a cumulative value of 35 for tomatoes planted before 23 May and 45 for tomatoes planted after 23 May. Subsequent sprays are applied after the accumulation of a predetermined DSV threshold since the last fungicide application. The last fungicide application should be made 14 days prior to harvest in fields with no recent history (last 2 years) of tomatoes and with a low incidence of disease or, 10 days in fields following minimal or no crop rotation (Pitblado 1992).

Simple data loggers, such as the Omnidata DP-223 (Omnidata Co. Logan UT) that measure leaf wetness and hourly average temperature, the parameters used to calculate DSVs, can be deployed in tomato fields to provide regional or local spray application recommendations. This enhances the potential of Tom-Cast to be regionally deployed for the control of fungal pathogens.

C. IMPORTANT PATHOSYSTEMS IN THE NORTHCENTRAL TOMATO PRODUCTION REGION

A pathosystem is defined as a component of a crop production system comprised of a host and any sub-set of pathogen(s) (Robinson 1976). Specific tomato pathogens encountered in the northcentral production region can be grouped into pathosystems based on their biology, epidemiology and disease management strategies. One pathosystem is comprised of *Verticillium* and *Fusarium* wilt, soilborne fungi that are effectively controlled through genetic resistance deployed in commercial cultivars. Two other important pathosystems and studied in this thesis include the fungal foliar-fruit pathosystem and the bacterial pathosystem.

1. FUNGAL FOLIAR-FRUIT PATHOSYSTEM

Economically important diseases that affect the foliage and fruit of tomato include early blight, anthracnose and *Rhizoctonia* soil rot.

a. EARLY BLIGHT

The early blight (EB) pathogen was first isolated in New Jersey in 1882 from dying potato (*Solanum tuberosum* L.) leaves (Ellis and Martin 1882). In 1892 the same fungus was shown to be a pathogen of tomato. Several binomials occur in the literature but the most common is *Alternaria solani* with the authorities (Ellis & Martin) Sorauer or, (Ellis & Martin) Jones & Grout. The literature pertaining to *A. solani* is extensive and has recently been reviewed by Pscheidt and Stevenson (1986).

Early blight is widespread in tropical and temperate zones (Ellis & Gibson 1975) and occurs wherever tomatoes are grown (Rands 1917; Jones et al. 1991). Early blight is particularly destructive in temperate humid climates such as the northcentral region and semi-arid climates where nightly dew is frequent and moisture requirements favor disease development (Sherf & MacNab 1986; Rotem & Reichert 1964).

Premature defoliation of tomato leaves is the primary effect of EB. Resultant impact on fruit yield and quality varies with environmental conditions, cultivar grown, geographic location, amount and time of arrival of inoculum, and defoliation severity (Basu 1974; Brammall 1993; Horsfall & Heuberger 1942; O'Leary 1985; Shoemaker 1976; 1980). Losses in marketable yield of fresh market tomato in North Carolina and in the absence of fungicide applications can be as high as 70% (Shoemaker 1976; 1980). In contrast, in Ontario during the 1991 and 1992 growing seasons, marketable yield of 13 fresh market tomato cultivars was not affected by EB epidemics even in the absence of fungicide sprays (Brammall 1993). Tomato plants can tolerate significant levels of defoliation before yields are detrimentally affected (Basu 1974; Ferrandino & Elmer 1992).

Yields of processing tomato in the midwest have been reduced up to 35% in

the absence of control (Sherf & MacNab 1986). Aesthetic appearance is not critical with processing as compared with fresh market tomatoes, but high mold counts depress the acceptibility and price of tomatoes at the processing plant (Precheur et al. 1992).

i. SYMPTOMS

Alternaria solani attacks all above ground tissue (Sherf & MacNab 1986) and has recently been reported to incite a root rot (Patterson 1991). Above ground symptoms have been described as collar rot and early blight.

Collar rot is not common in the northcentral production region but is more commonly associated with the southern production of tomato seedlings in open fields (Moore 1942; Pritchard and Porte 1921). Seedlings develop dark, sunken stem lesions close to the soil line and lesions can expand to girdle and kill plants or decrease productivity (Jones et al. 1991). Leaf spot symptoms are diagnostic for early blight. Symptoms are first observed on the lower foliage but progress to the upper foliage as the plant matures. Complete defoliation ensues in unchecked epidemics. Lesions first appear as small brownish black spots that expand up to 2 cm or more in diameter or become angular when restricted by leaf veins. Spots develop a series of concentric dark rings that give a characteristic "target spot" appearance. Spots are often surrounded by chlorotic tissue associated with phytotoxin production. As spots enlarge and coalesce infected leaves wither and abscise. Extensive defoliation can result in sunscald injury to fruit (Sherf & MacNab 1986).

Stems, branches and petioles can also be affected. Lesions are generally circular to elliptical with concentric rings. Expanded lesions can girdle stems and

weaken or wither apical portions of the tissue. Fruit are affected primarily at the stem end of the fruit inciting stem end rot (Horsfall & Heurberger 1942). The fungus appears to invade fruit from the calyx or pedicle and radiates through the stem end of the fruit to form concentric rings. The infected area is dry, leathery and firm and may be covered by a velvety mass of black spores (Jones et al. 1991; Sherf & MacNab 1986). Numerous small (<3 mm) black lesions on fruit and not restricted to the stem end have also been associated with *A. solani* (Thomas 1944).

ii. CAUSAL ORGANISM

A. solani is classified in the subdivision Deuteromycotina (the imperfect fungi), class Hyphomycetes, order Hyphales (Agrios 1988). A teleomorphic stage (*Pleospora solani* sp. nov.) has been reported (Esquivel 1984) but apparently is not common. Susceptible hosts, in addition to tomato and potato, include eggplant (*Solanum melongena* L.) and various solanaceous weeds such as horse nettle (*Solanum carolinense*) and black nightshade (*Solanum nigrum* L.) (Sherf & MacNab 1986; Rands 1917). *A. solani* expresses considerable variability in morphology, physiology and pathogenicity from one isolate to another (Bonde 1929; Henning & Alexander 1959; Wellman 1943). Although the presence of physiological races has been suggested, no differential host lines are known to substantiate this. The genetic variability of the pathogen, and ultimately how an understanding of the variability may impact disease management programs, is virtually unknown. Petrunak and Christ (1992) studied protein polymorphism using isozyme analysis and demonstrated a country (USA) wide sampling of *A.solani* isolates were distinct from *A. alternata* isolates collected from the same host (tomato or potato). The percentage of

polymorphic loci detected in the *Alternaria* isolates examined was 92%, considerably greater than levels observed for other fungi (Petrunak and Christ 1992).

iii. DISEASE CYCLE AND EPIDEMIOLOGY

A. solani is a multicyclic disease. The pathogen is able to overwinter in soil in association with plant debris as mycelium, conidia or chlamydospores (Basu 1971; Patterson 1991). Inoculum can persist on the surface or buried in the absence of host residue (Basu 1971). In Wisconsin, Rands (1917) demonstrated overwinter survival of the fungus in infected leaves and survival increased with depth of burial. Patterson (1991) noted incidence of collar rot decreased with increased inoculum depth. The relative persistence of inoculum is not known but chlamydospores are thought be the most important means of survival of primary soilborne inoculum (Patterson 1991). However, the pathogen's association with weeds (Rands 1917), seed (Moore et al. 1943) and transplants (Moore 1942) cannot be disregarded as sources of initial inoculum.

Spore germination occurs within 1 hr under optimum conditions. Free water or a relative humidity greater than 92% is required for germination (Stevenson & Pennypacker 1988). Penetration is direct, through wounds or stomata and occurs within 6 to 12 hr (Waggoner & Horsfall 1969). Infection increases with temperatures between 12 and 25° C (Moore 1942; Pound 1951). Lesions expand on mature tissue when the tissue is wet (Waggoner & Horsfall 1969). Infection efficiency increases with the load of fruit on the plant (Horsfall & Heuberger 1942; Waggoner & Horsfall 1969). Sporulation does not begin until lesions attain a size of 3 to 4 mm in diameter (Rands 1917). Conidiophore development occurs in the presence of free moisture and

is triggered by light (Waggoner & Horsfall 1969). Spore formation is favored on leaves first exposed to a dry light period and then a dark wet period (Rotem & Bashi 1969). The diurnal periodicity results in spore release and dissemination during the day if wind speed is sufficient (Harrison et al. 1965b; Rotem 1964). Somewhat paradoxically, spore germination and survival is favoured by the dark and inhibited by light (Stevenson & Pennypacker 1988; Rotem et al. 1985). Spore dispersal peaks soon after first lesions are detectable in mid to late July (Harrison et al. 1965b; Madden et al. 1978). Few spores are present prior to this peak and spore numbers are variable after this peak. Lesions can produce up to four crops of spores (Rand 1917) and under optimum conditions, 5 to 7 days are required from inoculation to production of spores.

In addition to environment, early blight progress is favored by low nitrogen (Thomas 1948; Horsfall & Heuberger 1942), early host maturity (Barratt & Richards 1944), nematode populations (Barker 1972), wounding (Rotem 1965), soil moisture stress (Rotem 1969) or other forms of stress associated with enhanced maturation or senescence of tissue.

In summary, *A. solani* effectively overwinters in temperate climates or is introduced to incite foliar epidemics. Disease initiates on mature tissue. Temperatures between 13 and 27°C and leaf wetness or high RH favor spore germination, penetration, lesion expansion and sporulation. Dry windy conditions favor dispersal. Factors that enhance host maturity or senescence enhance disease incidence.

iv. CONTROL

Despite an extensive literature reporting research results on the epidemiology, etiology and control of the pathogen, early blight is one of the primary diseases of

tomato targeted in routine fungicide applications in the northcentral production region. Extensive surveys and subsequent work have identified sources of resistance to *A. solani* (Alexander et al. 1942; Barksdale & Stoner 1977; Gardner 1988; Maiero et al. 1990a; Nash & Gardner 1988a). However, inheritance of resistance is complex (Nash & Gardner 1988b; Maeiro et al. 1989). No commercial cultivars, processing nor fresh market, have acceptable levels of resistance to early blight. O'Leary (1985) has shown that resistance in some of the most promising lines is a form of horizontal resistance or rate reducing resistance governed by the interaction of infection efficiency, lesion area, latent period, sporulation capacity per lesion and sporulation capacity per unit area.

O'Leary (1985) has demonstrated that levels of resistance can be combined with a reduced fungicide spray program to achieve control of early blight.

Basu (1974) fumigated infested soil and decreased initial levels of early blight but growth and yield of tomato plants was not affected. A 2 to 3 year rotation to decrease initial inoculum has also been suggested (Horsfall & Heurnerger 1942; Rands 1917; Sherf & MacNab 1986) but limited experimental evidence is available to assess the impact of rotation.

Nitrogen applications, up to a certain optimum, delay plant maturity and reduce incidence of early blight (Fischer 1986; Horsfall & Heurberger 1942; Jones & Jones 1986; Thomas 1948). Delayed senescence associated with nitrogen appears to decrease the apparent infection rate of disease progress (Fischer 1986; Mackenzie 1981). Mulch around plants can have a positive affect depending on the year (Fischer 1986).

The potential for biological control has received even less attention than cultural practices. Brame and Flood (1983) demonstrated a 2-day pre-incubation with

Aureobasidium pullulans on leaf surfaces significantly reduced infection and growth of *A. solani*. However, the antagonism could not be associated with inhibitory metabolites. Rather, *A. pullulans* incited host defense responses and this impacted infectivity of *A. solani* (Flood & Rees 1986). Casida and Lukezic (1992) demonstrated a *Pseudomonas* isolate, strain 679-2, provided a reduction in the severity of early blight in field trials. No adverse affects to the plant were noted and antagonism was associated with a water soluble inhibitory compound. Leben and Daft (1965) spray inoculated tomatoes with a bacteria, originally isolated as an epiphyte on cucumbers, and reduced early blight incidence when challenged 2 -3 days later.

Multiple disease control strategies have been researched but few have progressed sufficiently far to offer adequate control of early blight. Fungicides continue to be the primary and often only strategy for control of early blight.

b. ANTHRACNOSE

A fungus associated with anthracnose was first described in 1879 by Saccardo (Sherf & MacNab 1986). Anthracnose has been recorded in Asia, Europe, Africa, the East Indies and North America (Jones et al. 1991). In North America, anthracnose is an economic problem in the northeast and midwest U.S.A. and central Canada. The literature has recently been reviewed by Dillard (1992).

Anthracnose is primarily a disease of ripe fruit and is the most important fruit rot disease of processing tomatoes in the northcentral production region (Barksdale & Stoner 1981; Preucher et al. 1992; Stevenson et al. 1978). Disease incidence can be as high as 70% (Wilson & Runnels 1949) in the absence of control and 5 to 15 % even with repeated applications of fungicide (Sherf & MacNab 1986). Amonut of disease is

a function of the production system. Harvest of ripe fruit for processing is often delayed for extended periods to allow optimum timing for once-over machine harvesting. In contrast, fresh market tomatoes are picked in multiple harvests at the breaker stage (first appearance of color) and are shipped and often consumed before latent infections develop into lesions.

i. SYMPTOMS

Symptoms appear only on on ripe or senescent fruit (Dillard 1992; Jones et al. 1991; Scherf & MacNab 1986). Lesions on ripe fruit first appear as light brown flecks and expand as sunken circular lesions up to 12 mm in diameter. Tissue in the center of the lesion darken and contain acervuli that bear masses of salmon-colored spores during wet weather. Large portions of the fruit become rotted as lesions expand and coalesce and secondary organisms invade the tissue.

Leaves, stems and roots may also be affected. Leaf symptoms are rare but can appear 5 to 7 days after inoculation and enlarge to a maximum size of 2 mm. Leaf lesions are sunken, necrotic in the center and may be surrounded by a halo (Younkin & Dimock 1944). Root symptoms include black dots (microsclerotia of the fungus), brown lesions or brown root rot.

ii. CAUSAL ORGANISM

Colletotrichum coccodes (Wallr.) Hughes is the most common species associated with anthracnose fruit rot but numerous other species can infect tomato fruit (Batson and Roy 1982; Stevenson et al. 1978). Black dot disease of tomato roots is also associated with *C. coccodes* but is not a known concern in the northcentral

production region.

C. coccodes is classified in the subdivision Deuteromycotina (the imperfect fungi), class Coelomycetes, order Melanconiales (Agrios 1988). *C. coccodes* isolates vary considerably in pathogenicity, growth rates, pigmentation, sclerotia size and in other characteristics (Dillard 1992; Sherf & MacNab 1986). No details of population diversity is known. *C. coccodes* has a wide host range including members of the *Solanaceae, Leguminaceae* and *Cucurbitaceae* family. Over 19 families comprising 68 species, including numerous weeds, have been identified as hosts (Jones et al. 1991).

iii. DISEASE CYCLE AND EPIDEMIOLOGY

Inoculum inciting fruit rot may originate from overwintered crop debris, alternative hosts or secondary inoculum from other infected plant tissue on other portions of the plant.

Acervuli on infected tissue give rise to sclerotia, the primary mechanism of overwintering and survival for the fungus (Dillard 1992). Inoculum persists in association with overwintering tomato skin tissue (Dillard 1990; Farley 1972). Dillard (1990; 1992) buried colonized skins of tomato fruits 0, 10 and 20 cm deep and after 3 years found 70% of tissue harbored viable propagules and 90% of the sclerotia were viable.

Weeds or other crops can serve as hosts and as a source of primary inoculum to susceptible crops (Batson & Roy 1982; Raid & Pennypacker 1987). Komm & Stevenson (1978) observed incidence of *C. coccodes* propagules and disease incidence on potato was considerably less in a reclaimed forest land as compared to a field with a history of potato production. However, virgin forest soils did harbor inoculum and

incite disease. Inoculum levels increase in the absence of rotation.

Younkin & Dimock (1944) experimentally demonstrated that *C. coccodes* is able to infect foliage. Leaf lesions on attached leaves can sporulate but not abundantly (Younkin & Dimock 1944). Farmer (1959) and Illman et al. (1959) demonstrated detached and senescent tissue supported profuse colonization and sporulation. Pantidou and Shroeder (1955) reported leaves can be infected, especially lower leaves, in the field and provide secondary inoculum to fruit. Wounding (sand-blasting) and extended post-inoculation wetting periods significantly increase infection and lesion development on potato foliage (Johnson & Miliczky 1993). The relative importance and relationship of foliage infection, importance of foliage as a source of secondary inoculum, the environmental conditions favoring leaf infection and final incidence of tomato fruit rot, is largely unknown. Work is currently in progress to address these issues (Hausbeck & Linderman 1992).

Growth from sclerotia is optimum at 28°C (Dillard 1988) and favored by wet conditions. Sclerotia germinate by producing hyphae or by producing conidia in acervuli on the sclerotia surface.

Inoculum may come in contact with fruit resting on the soil or be splashed by rain onto susceptible surfaces. Fruit in various stages of development are susceptible to direct penetration in the absence of wounding. Infections of green fruit are latent (Farmer 1959; Fulton 1948) and as the fruit ripens, symptoms develop rapidly. Symptoms develop rapidly on inoculated ripe fruit. Germination of conidia occurs at an optimum temperature of 22°C and a minimum of 10 hr of continuous wetness is required for infection. Lesion expansion is favored by temperatures 16 to 31°C and subsequent conidia formation increases with increasing temperature from 16 to 28°C

(Dillard 1988; 1989).

Determinate and open growth, early maturity and early defoliation has been associated with increased levels of anthracnose on fruit (Wilson & Runnels 1949). Barksdale & Koch (1969) demonstrated soil type impacts disease incidence. They recorded almost twice the general amount of natural infection on plants grown in sandy soil as compared to those grown in clay soil.

In summary, *C. coccodes* is associated with numerous hosts, including weeds common in the northcentral production region. Sclerotia can persist in association with host debris for 3 or more years and is the primary source of initial inoculum. Disease progress is favored by warm temperatures (24 - 28°C) and wet conditions.

iv. CONTROL

Control recommendations include crop rotations of 3 to 4 years that exclude known susceptible hosts (e.g. potatoes), adequate control of weed hosts, timely harvest of ripe fruit, use of tolerant genotypes and routine applications of fungicides.

Few studies have been conducted to determine the impact of crop rotation and weed management programs. Precise timing of machine harvesting and the use of ethephon to speed up ripening help reduce anthracnose levels (Sherf & MacNab 1986).

Genetic resistance is available in advanced determinate breeding lines that have yield and fruit qualities similar to commercial lines (Barksdale & Stoner 1981). Breeding lines exhibited 87 - 99% less anthracnose during natural infection as compared to a susceptible control (Stevenson et al. 1978). Although resistance is controlled by a number of genes, Miller et al. (1984) concluded relatively rapid genetic advance should be possible in breeding and selection of resistant genotypes.

Anthracnose resistance incorporated in the advanced breeding lines offered control comparable to susceptible controls but with 3 to 7 fewer fungicide sprays (Barksdale & Stoner 1981).

Breeding for disease resistance is complicated by the ability of multiple species able to incite anthracnose fruit rot and the variability of virulence among isolates within each species (Batson & Roy 1982). However, resistance to one species may be correlated with resistance to other species (Barksdale 1972; Stevenson et al. 1978). The relative importance of the various species in field incidence of anthracnose is not known.

Currently, anthracnose is controlled through the routine application of protective fungicides. Because the anthracnose fungi can infect green fruit, fungicide recommendations call for an initial spray when fruit first set.

Anthracnose was reduced considerably with the application of 14 pounds/acre of maneb (Dithane M-22) directly to the soil in late June. Three foliar sprays of maneb during the season enhanced control (Crossan et al. 1963).

Leben and Daft (1965) evaluated the potential of biological control. They spray inoculated tomatoes with a bacteria, originally isolated as an epiphyte on cucumbers, 2 days prior to a challenge inoculation with the anthracnose fungus. Anthracnose incidence was reduced.

c. RHIZOCTONIA SOIL ROT

Rhizoctonia solani was first observed as a pathogen of potato in 1858 by Julius Kuehn. *Rhizoctonia* appears to have an unlimited host range and is studied in hundreds of research programs (Parmeter 1970). The pathogen attacks tomato worldwide and is

able to incite damping off, root rot, stem canker and fruit rot. In field production systems, *Rhizoctonia* soil rot is the principal component of the soil rot disease complex of tomato fruit and can result in losses up to 75% (Batson 1973; Jones & McCarter 1974).

i. SYMPTOMS

Soil rot may affect fruit at any stage of development but is most common on ripening fruit at or near the soil. Lesions commence as small, firm brown spots that progressively enlarge. Lesions expand rapidly, may have concentric zones and become soft and mushy as the pathogen ramifies through the tissue and secondary organisms move in. The pathogen may also cause damping off or poor plant productivity of young plants early in the season.

ii. CAUSAL ORGANISM

Rhizoctonia solani Kuehn is a heterogenous collection of strains that vary considerably in pathogenicity, culture characteristics and saprophytic ability. The species has sterile mycelia (no spores) and is generally divided into subgroups based on anastomosis behavior. The pathogen is ubiquitous and able to attack most plant species. It is classified in the subdivision Deuteromycotina (the imperfect fungi), class Agonomycetes (Mycelia Sterilia), order Agonomycetales (Agrios 1988). The teleomorph is classified in the subdivision Basidiomycotina.

iii. DISEASE CYCLE AND EPIDEMIOLOGY

Papavisas et al. (1975) demonstrated in Maryland that saprophytic activity of *R. solani* peaks in the top 10 cm of the soil and soon after soil incorporation of crop residue. Soil populations declined rapidly, following conventional tillage practices, to relatively low numbers by the following spring. *R. solani* is highly dependent on plant tissue and disappears with the reduction of food bases (Papavisas et al. 1975). Persistence in the soil is dependent on the strain (Carling and Leiner 1990; Parmeter 1970). Penetration is direct with an optimum temperature for infection of 25°C and a requirement for free moisture or high relative humidity (Gonzalez & Owen 1963). Once successful invasion occurs, lesion expansion is not limited by moisture.

iv. CONTROL

Control recommendations include staking fresh market tomato varieties or using plastic or paper mulch (Jones & McCarter 1974) to limit fruit contact with soil. Staking is labor intensive and recent reports indicate a move away from this horticultural practice. Straw mulch reduces soil rot but affects are variable with location (Jones & McCarter 1974). Staking or use of mulch is not practical in processing tomato production.

Polygenic resistance, useful for processing cultivars, has been identified (Barksdale 1974).

Fungicide applications at regular intervals during fruit development can be moderately effective in providing control (McCarter & Barksdale 1977) but are not considered to be economical for fruit rot control (Jones et al. 1991). Alternatively, Crossan et al. (1963) showed soil rot was reduced considerably with the application of

14 pounds/acre of maneb (Dithane M-22) directly to the soil in late June. Three foliar sprays of maneb during the season did not increase disease control. The economics of the later approach is questionable (McCarter and Barksdale 1977) and is not practiced.

Because the pathogen survives in colonized plant debris, it is influenced more by tillage practices than many other soilborne pathogen (Sumner et al. 1986a). The pathogen does not persist well at depths of 5 - 10 or more cm (Papavisas et al. 1975). Moldboard plowing and burial of crop debris has greatest benefit where high inoculum levels of *R. solani* exist (Sumner et al. 1986a). Root disease severity is probably influenced more by the previous crop however, than by tillage system (Sumner et al. 1986b; Rush & Winter 1990).

Lewis et al. (1990) evaluated the potential to biologically control *R. solani* with a *Trichoderma* and *Gliocladium* isolate. After 5 years of greenhouse and field evaluation, the authors concluded some control was possible in the greenhouse but this form of biological control was not useful in field production. Limitations to successful control included the ubiquitous nature of *R. solani* having broad ecological capabilities, extended periods of time associated with disease progress and the complexity of environmental, physical and biological factors that impacted pathogen-biocontrol agent interactions.

d. FUNGAL FOLIAR-FRUIT PATHOSYSTEM:SUMMARY

The epidemiology, etiology and biology of early blight and anthracnose has not been systematically compared. However, a review of the literature demonstrates that each disease can be endemic and is favored by similar environmental conditions and host maturity. The life cycle and epidemiology of *R. solani* differs considerably

but is also endemic and the target of routine fungicide applications.

2. THE BACTERIAL PATHOSYSTEM

a. BACTERIAL CANKER, BACTERIAL SPOT AND BACTERIAL SPECK

Bacterial canker, bacterial spot and bacterial speck, are economically important bacterial diseases throughout the northcentral production region. Each disease occurs yearly but sporadically, not occurring on every farm every year, and is differentially affected by the environment and crop production system. However, the pathogens that cause canker, speck and spot share many biological and epidemiological features and comprise a pathosystem.

Bacterial canker, spot and speck were first reported in 1910, 1920 and 1933, respectively (Bryan 1933; Doidge 1921; Smith 1910) and occur worldwide (Jones et al. 1991; Sherf and MacNab 1986). Canker is one of the most destructive diseases of tomato but each disease can be responsible for up to 70% loss in yield or fruit quality (Pohronezny and Volin 1983; Strider 1969; Sherf and MacNab 1986; Yunis et al. 1980). Marketable yield loss is associated with defoliation and fruit lesions with all three diseases and early season wilt in the case of canker. Lesions on fruit limit sales of fresh market tomato and hinder skin removal and product quality of processing tomato.

i. SYMPTOMS

A wide array of symptoms, that can be categorized as two separate phases, are associated with bacterial canker (Gleason et al. 1993; Strider 1969). A systemic phase, is initially manifested early in the season as unilateral wilting of leaves but eventually,

the entire plant may wilt or develop stem cankers and die. A second phase is diagnosed initially as firing of leaf margins. Necrosis and wilting of entire stems ensue as bacteria basipetally migrate into the tissue. Fruit lesions are raised brown spots often surrounded by a yellow halo giving the appearance of a "bird's eye", a diagnostic feature of canker.

Spot may incite blighting of leaves associated with coalescing of numerous lesions. All above ground parts of the plant can be affected and individual lesions are dark and rarely larger than 3 mm. Fruit spots are 2 to 10 mm in diameter, initially appearing as small water soaked areas but later turning brown to gray. Subepidermal infections result in a scabby appearance as surface tissue disintegrates. Speck, like spot, is able to affect all above ground portions of the plant. Lesions are small, no larger than one mm. Many lesions may occur on infected tissue and coronitine, a phytotoxin produced by the speck pathogen, dissipates to incite large areas of chlorosis. Superficial and slightly protruding black lesions form subepidermally on fruit. Lesions may coalesce and affect large expanses of the fruit.

ii. CAUSAL ORGANISM

Bacterial canker is caused by *Clavibacter michiganensis* subsp. michiganensis (Smith) Davis et al. (Cmm), a gram positive, non-spore forming pleomorphic bacterium. Differences in pathogenicity have been reported (de Vries 1990) but the genetic diversity of the pathogen is not known.

Bacterial speck is caused by *Pseudomonas syringae* pv. tomato (Okabe) Young; Dye & Wilkie (Pst), a motile gram negative rod. The pathogen appears to be comprised of two closely related lineages (Cooksey & Graham 1989; Denny et al.

1988). Two races have been identified based on virulence for differential hosts (Lawton & McNeill 1986).

Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv), a motile, gram negative rod shaped bacterium, incites bacterial spot on tomato and pepper. The pathovar is phenotypically (Dye et al. 1964), serologically (Jones et al. 1993b), pathogenically (Minsavage et al. 1990) and genotypically diverse (Vauterin et al. 1990). The pathovar has been described with the ability (Dowson 1949), general inability (Gitaitis et al. 1987) or variable ability (Dye 1964) to hydrolyse starch. In North America, primarily Georgia and Florida, where most research on Xcv occurs, a starch negative reaction is considered diagnostic (Gitaitis et al. 1987). Starch positive strains associated with tomato plants in Georgia, have been shown to be non-pathogenic opportunistic epiphytes (Gitaitis et al. 1987). Likewise, pectolytic and non-pectolytic isolates are known to exist. Beaulieu et al. (1991) concluded pectolytic activity was correlated with the geographical origin of isolates. For example, 90% of isolates obtained from Argentina were pectolytic as compared to only 1 of 374 isolates originating from the United States.

Minsavage et al. (1990) categorized Xcv isolates into groups and races based on virulence for pepper and tomato genotypes. The pepper group was subdivided into 3 races. Race 1 has virulence for pepper only and race 2 and 3 have virulence for tomato plants and selected pepper genotypes. Typing of pepper races is based on differential reaction on near isogenic pepper lines with an interaction that functions in a gene-for-gene manner. Minsavage et al. (1990) designated a tomato group of strains, comprised of one race (T1), based on ability to infect tomato genotypes and no known pepper genotypes. More recently, a second tomato race (T2) has been identified (Wang

et al. 1990). T2 strains, also designated "B" strains, are pectolytic and/or starch hydrolytic and serologically distinct as compared to T1 or "A" strains (Jones et al. 1993).

iii. DISEASE CYCLE AND EPIDEMIOLOGY

Cmm, Pst and Xcv are able to overwinter in association with crop debris, weeds and volunteer plants (Chang et al. 1992; Gleason et al. 1991; Jardine et al. 1988; Peterson 1963). Overwintered inoculum may serve as a source of inoculum to subsequent tomato crops (Chang et al. 1992; Gleason et al. 1991; Jardine et al. 1988). The bacteria are poor saprophytes and do not persist in soils for long periods of time in the absence of host debris (Gleason et al. 1991; Jones et al. 1991). Overwintered sources of inoculum appear to lead to epiphytic populations on tomato leaf surfaces and disease symptoms appear mid to late season after a threshold of 10^6 cfu per leaflet is achieved (Gleason et al. 1991). All three bacteria are known to be seedborne (Dhanvantari 1989; Gardner and Kendrick 1921; McCarter et al. 1983; Sijam et al. 1991). Contaminated seed is the most important source of inoculum (Dhanvantari 1989) for bacterial canker. Seed borne inoculum gives rise to systemically infected seedlings that often remain symptomless for up to 8 wks after field setting. Wilt and symptoms of primary canker are enhanced by stress conditions such as low moisture.

The importance of Pst inoculum on seed is supported by a worldwide outbreak of the disease that occurred in 1978 (Goode and Sasser 1980). In the case of Xcv, the relative importance of seedborne inoculum, especially in the northcentral region, is unknown. Using current screening procedures, the frequency of contaminated tomato seedlots is reportedly low.

Secondary spread of all three bacteria occurs by handling of transplants prior to field setting, by splash dispersal, insects and field production activities (Bashan 1986; Chang et al. 1991; McCarter et al. 1983; McInnes et al. 1988; Volcani 1969). Optimum disease progress is enhanced by wet conditions and temperatures of 25° to 32°, 18° to 24° and 24° to 30°C for Cmm, Pst and Xcv, respectively (Jones et al. 1991; Sherf and MacNab 1986). Infection may occur through stomata but is favored by wounds inflicted by insects, cultural practices, wind or sandblasting damage (Getz et al. 1983b; Carlton et al. 1992; Vakili 1967). Fruit infection occurs through natural wounds and when fruit is less than 3 mm in diameter (Getz et al. 1983a).

iv. CONTROL

Prevention is the most important control strategy for controlling bacterial canker, speck and spot (Goode and Sasser 1980; Gitaitis et al. 1992). Zero tolerance in seedlots is the goal of the industry but has proven unrealistic due to the limits of detection and seed treatment technologies (Sasser and Goode 1980). For example, Cmm seed infection levels less than 0.1% are difficult to detect using current seed plating assays (Dhanvantari 1989) but as few as 0.01% infected seedlings, grown according to specific cultural practices, can initiate a serious epidemic in tomato plantings (Chang et al. 1991; Gitaitis and Beaver 1991).

Currently, samples of tomato seeds are ground and the extract plated on selective media (Gitaitis et al. 1992). Suspect colonies are evaluated by selected phenotypic tests, ELISA, fatty acid profile analysis, or induction of a hypersensitive response on a non-host (Gitaitis et al. 1992). Each presumptive diagnosis is followed by pathogenicity tests. No protocols are available for rapid, non-presumptive

identification of the bacteria. Diagnostic probes specific for Pst (Cuppels et al. 1990) and Cmm (Thompson et al. 1989) have been developed but not commercially deployed.

Sanitation in greenhouse production of transplants and around the field (e.g. removal of weed hosts and volunteers) is important (Sherf and MacNab 1986). Crop rotations are routinely recommended (Jones et al. 1991). Gleason et al. (1991) and Jardine et al. (1988) have demonstrated burial of infected host debris enhances decline of overwintered inoculum. Thus, fall plowing is also often recommended. Other cultural practices include the use of windbreaks to decrease sand blast injury, and avoidance of activities that wound tomato plants.

Chemical based control generally has not proven effective though often used. Jardine and Stephens (1987) demonstrated applications of bactericides were effective only when conditions for disease were limiting. Yunis et al. (1980) applied copper sprays weekly and reduced speck disease severity. However, routine use of streptomycin or copper has led to bacteria populations resistant to the chemicals (Bender and Cooksey 1986; Marco and Stall 1983; Stall and Thyer 1962). Bactericides must be applied within 24 - 48 hrs post infection and on a 4 to 7 day schedule if they are to have any utility (Jardine and Stephens 1987). Routine fungicide applications do not control the bacteria (MacNab 1980).

No commercial cultivars have acceptable levels of genetic resistance despite extensive surveys of domesticated and wild germplasm (Alexander 1942; Crill et al. 1972; Lawson and Summers 1984a & b; Pilowsky and Zutra 1982; Scott and Jones 1986; Thyr 1968). Resistance to Cmm is complex and not easily transferred to commercial cultivars (Jong and Honma 1976). Pitblado and Kerr (1979) identified a

tomato genotype with vertical resistance (Pto gene) to Pst. However, Lawton and McNeill (1986) demonstrated a virulent race was present at low levels in natural populations even before the resistance gene was deployed. Likewise, Scott and Jones (1986) identified a tomato genotype, Hawaii 7998, with resistance to Xcv. However, before Hawaii 7998 was generally deployed, virulent strains from Argentina were identified (Wang et al. 1990).

b. SUMMARY OF BACTERIAL PATHOSYSTEM

Bacterial canker, spot and speck are important diseases in the northcentral production region. Cultural practices, including crop rotation and routine chemical sprays have not prevented marketable yield-reducing epidemics. Genetic resistance is currently not deployed and sources of resistance have not proven durable or easy to incorporate into commercial cultivars. Prevention is the key to control. However, detection assays are not sensitive enough to detect epidemiologically significant seedborne populations. Advances in developing specific detection protocols and implementing breeding programs have not been informed by an understanding of the chromosomal based genetic diversity of each pathogen.

D. ASSESSING POPULATION GENOTYPIC DIVERSITY OF PLANT PATHOGENS

Chromosome based assessment of genetic diversity has been limited by technology. Recently, multilocus enzyme electrophoresis (MLEE) (Denny et al. 1988), restriction enzyme digestion of total DNA (Cooksey and Graham 1989; Hartung and

Civerelo 1987), and restriction fragment length polymorphism (RFLP) analyses with specific probes (Berthier et al. 1993; Denny et al. 1988; Leach et al. 1992; Levy et al. 1991) have been utilized to assess genetic diversity of plant pathogens. Alternatively, polymerase chain reaction (PCR) based protocols using arbitrary primers (Welsh and McClelland 1990; Williams et al. 1990), primers corresponding to t-RNA (Welsh and McClelland 1991), or primers corresponding to 16S and 23S genes (Jensen et al. 1993) have been used to discern difference among strains of bacteria. The potential of each protocol to delineate genotypic diversity of plant bacteria has not been established.

This thesis explores the potential of yet another PCR-based approach, known as rep-PCR and recently reviewed by Lupski and Weinstock (1992). Families of repetitive sequences are dispersed throughout the genome of diverse bacterial species (Versalovic et al. 1991; Koeuth et al. 1994). Three families, though not related by DNA sequence homology, include the 35-40 bp repetitive extragenic palindromic (REP) sequence (Gilson et al. 1984; Higgins et al. 1982), the 124-127 bp enterobacterial repetitive intergenic consensus sequence (ERIC) (Hulton et al. 1991; Sharples and Lloyd 1990) and the recently discovered 154 bp BOX elements (Martin et al. 1992). Primers corresponding to the repeated palindromic sequences anneal to DNA via PCR and DNA between two adjacent sequences is amplified according to the processing limits of the Taq polymerase enzyme. PCR products are separated on agarose gels and provide species and strain specific banding patterns (Versalovic et al. 1991; de Bruijn 1992; Koeuth et al. 1993).

The relative potential of rep-PCR to assess genotypic diversity has not been fully determined. Patterns of similarity in rep-PCR banding patterns correspond to phylogenetic relationships determined by MLEE (de Bruijn 1992) and RFLP analyses

(Judd et al. 1993).

In the case of MLEE, variation in mobility of proteins can be directly associated with alleles of known genes. Variation appears to be selectively neutral, and can be scored to statistically determine genetic diversity among bacterial isolates and phylogenetic relationships among lineages (i.e.clones) can be ascertained (Selander and Musser 1990). In contrast, polymorphism in rep-PCR patterns can not be equated to single loci nor can such polymorphisms be assumed neutral. To the contrary, rep-like sequences may have a functional role (Lupski and Weinstein 1992) and are highly constrained over time within pathogenic clones (Woods et al. 1992). Rep-like sequences may in fact have a role in genome organization and niche specialization (Kraweic 1985). A theoretical framework for determining genetic distance and evolutionary structure of bacteria using rep-PCR has not yet been developed.

Thorough sampling of genotypic diversity of clinical and animal pathogens has provided a framework to assort pathogenic populations with respect to host species, geographic distribution and nature of disease caused (Selander and Musser 1990). Likewise, proposed disease specific virulence factors can be associated with specific clonal groups and disease specificity (Achtman and Pluschke 1986; Selander and Musser 1990). Similar sorting of bacteria may be possible using rep-PCR and may be useful for identifying true pathogenic variants and elucidating the diversity of bacterial pathogens and symbionts (de Bruijn 1992; Judd et al. 1993; Versalovic et al. 1993; Woods et al. 1992; Appendix D). The implication, similar to the case of pathogens of humans and animals (Selander and Musser 1990), is that the unit important to devising integrated disease management strategies to control plant pathogenic bacteria, is not the species, subspecies or pathovar, but the clone. A clone, or evolutionary lineage,

may or may not be coincident with taxonomical divisions.

A key question that plagues plant pathologists concerns the origin of pathogenic variants and the factors that govern host range. Dr. Gabriel's group (Swarup et al. 1991; 1992; Waney et al. 1991) have shown host-specific virulence (*hsv*) genes can function as positive factors to determine host range. Horizontal transfer (i.e. a recombinational mechanism of convergence) of *hsv* gene(s) could give rise to clonal groups with distinctive chromosomal genotypes but similar host range. In contrast, Stall et al. (1994) suggest that host range, for example in Xcv, "is determined by avirulence genes carried on plasmids". Kearney et al. (1988) suggest mutation of single genes give rise to virulent pathotypes. Indeed, avirulence genes cloned into foreign backgrounds alter host range (Kobayashi et al. 1989; Whalen et al. 1988). However, this may be a gratuitous function (Gabriel 1989) and a result of common ancestry of the pathogen or host (Heath 1991).

Emphasis on single genes and gene-for-gene models has been associated with a boom-and-bust cycle in genetic resistance breeding. Pathogenic variants, for example within Xcv, have traditionally been described in terms of race governed by single genes for avirulence that can be identified if genetic lines within a host species have differential sets of genes for resistance (Minsavage et al. 1990). However, avirulence genes may not necessarily be a component of basic compatibility (Heath 1991). This thesis attempts to use protocols that describe pathogenic diversity in terms of overall chromosomal organization using rep-PCR, and identify lineages or clones, primarily in Xcv, that may have arisen as a function of basic compatibility with tomato. Assessment of chromosomal organization has been useful to resolve pathotypes in other pathosystems (Leach et al. 1992; Levy et al. 1991). The potential to capitalize on

knowledge of genetic diversity has only begun to be explored and may provide a framework for understanding pathogenesis, evolutionary dynamics and optimal methods for the implementation of integrated disease management strategies, including the deployment of host resistance (Leach et al. 1992; Levy et al. 1991).

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CHAPTER II

INTEGRATED MANAGEMENT OF EARLY BLIGHT, ANTHRACNOSE AND SOIL ROT OF TOMATO WITH REDUCED FUNGICIDE USAGE AND CULTURAL PRACTICES

ABSTRACT

The integration of a reduced-sprays program and conservation tillage was studied in 1990 - 1992 in fresh market (FMT) and processing tomato (PRT) (*Lycopersicon esculentum*) production systems for the control of early blight (EB), caused by *Alternaria solani*, anthracnose (ANTH), caused by *Colletotrichum coccodes*, and soil rot (SR), caused by *Rhizoctonia solani*. Reduced-sprays were scheduled according to TOMCAST, a program that calculates a daily disease severity value (DSV) based on the average temperature during hours when tomato foliage is wet. Select forecast generated chlorothalonil spray schedules required 45-80% fewer applications but did not compromise incidence of percent PRT fruit with mold in 1990-1992 nor incidence of percent FMT fruit with mold in 1991-1992 as compared to a standard weekly spray program. Reduced-sprays did not adequately control a high incidence of SR on FMT fruit in 1990. Reduced sprays were most effective in disease control when integrated with a zone tillage (ZT) system in 1990, a conventional tillage (CT) system in the absence of rotation in 1991, and ZT or rotation in 1992. Zone tillage, as compared to CT, reduced mean area under the disease progress curve (AUDPC) due to EB in 1990 and in the absence of rotation in 1991, resulted in increased AUDPC values. In 1992, ZT decreased mean AUDPC values in plots planted to continuous tomato (no rotation) and increased mean AUDPC in tomato plots rotated to cucumber the preceding year. Integrated disease management of EB, ANTH and SR was possible with reduced fungicide input and cultural practices affording advantages associated with sustained productivity of farmland.

INTRODUCTION

Early blight, caused by *Alternaria solani* (Ellis & Martin) Jones & Grout, anthracnose (ANTH), caused by *Colletotrichum coccodes* (Wallr.) Hughes, and soil rot (SR), caused primarily by *Rhizoctonia solani* Kuehn (Batson 1973; Jones & McCarter 1974) are the most important fungal diseases of tomato foliage and fruit in the northcentral (NC) production region (MI, OH, IN, Ontario) of North America. High humidity and warm temperatures combined with extensive dew periods and abundant rainfall favor EB, ANTH and SR each season. Each disease can incite losses of 35 to 70 % or more (Batson 1973; Jones & McCarter 1974; Sherf and McNab 1986; Wilson and Runnels 1949). Therefore growers currently follow standard recommendations to initiate fungicide sprays when fruit first set and to apply subsequent applications every 7 to 14 days on nearly 100% of the tomato hectarage. Accordingly, twelve or more seasonal sprays are applied with limited consideration of disease level, weather patterns or cropping systems.

The recent removal of several fungicide products has currently put the tomato industry in the tenuous position of relying, almost exclusively, on only two families of fungicides, the ethylene-bis-dithiocarbamates (EBDCs) and chlorothalonil, for fungal disease control. Loss of either fungicide would impinge on the ability of the northcentral tomato region to reliably produce a marketable crop within the context of current production systems. In addition, fungicide usage is facing an unprecedented challenge including consumer, regulatory agency, environmental, grower-safety, and cost of application concerns. The loss of registered materials, residue concerns, and lack of forthcoming new chemistry requires prudent use of EBDCs and chlorothalonil

to minimize food safety concerns and ensure their long-term use until alternative disease control strategies can be devised and implemented.

The challenge tomato growers currently face is not limited to their desire to limit fungicide use. Conventional production systems with intense tillage of the land and energy-intensive inputs contribute to high variable production costs and are seen as counter-productive to a sustainable agricultural system (Fretz et al. 1993). At the farm enterprise and field level, components of sustainability include reduced tillage practices, maintenance of a surface crop residue, use of cover and green manure crops, and crop rotation (Fretz et al. 1993; Frye and Blevins 1989; Sarrantonio 1992; Shennan 1992). Sustainable farming practices have not been adopted with equal success in vegetable (Kelly 1990) as compared to field crop production systems (Phillips et al. 1980). In part, moldboard plowing and intensive tillage have been crucial cultural practices for breaking the life cycle of pests that limit vegetable productivity (Putnum 1990; Sumner et al. 1986). Advancements in reduced tillage and cover crop use have recently been made in numerous vegetable production systems (Coolman and Hoyt 1993; Morse 1993; Phatak 1992; Sarrantonio 1992; Shennan 1992; Wallace and Bellinder 1992; Wien 1990) including tomato (Abdul-Baki and Teasdale 1993; Doss et al. 1981; Knavel et al. 1977; McKeown et al. 1988; Price and Baughan 1988; Shelby et al. 1988). Unfortunately, most studies have not included a pathology component (Sumner et al. 1986) and cannot be recommended without a greater appreciation of the system as a whole.

The objective of this study was to reduce fungicide usage and evaluate the potential of integrated disease management practices for the control of early blight, anthracnose, and soil rot within the context of a fresh market (FMT) and processing

tomato (PRT) production system currently under research at Michigan State University. We validated a TOMato disease foreCASTing model, TOMCAST (Pitblado 1988), to determine the need for fungicide sprays and we studied the impact of reduced tillage, cover and green manure crops, and a one year rotation as they affect the foliar-fruit fungal disease complex.

MATERIALS AND METHODS

Location and design of field experiments. Field experiments were initiated in the fall of 1989 and conducted in 1990, 1991 and 1992 on a Spinks sandy loam (87.4% sand, 6.0% silt and 6.6% clay) at the Southwest Michigan Research and Extension Center near Niles, MI. The site of the experiment had been seeded to rye and was soil incorporated September 1989. Rye (*Secalis cereale* cv. Wheeler) was drilled at 168 kg ha^{-1} in the fall prior to each planting season. Inoculum was not introduced but presumably arose from indigenous sources or as transplant and seed borne inoculum.

The experiment had a split-split plot design with four replications arranged in randomized complete blocks. Each replication comprised two main plot parcels of land (about 12 x 84 m) side by side and separated by approximately 9 m of permanent sod. Each main plot was subdivided into two 12 x 42 m sub-plots and each subplot was divided into seven 12 x 6 m sub-sub-plots for a 2x2x7 factorial design (n=112). Each plot contained four cropped rows on 1.5 m centers. Plots planted to tomato (*Lycopersicum esculentum*) consisted of two rows of a fresh market cultivar and two rows of a processing cultivar. All data, were taken from the inner 6 m of each plot of the inner plot-row of each tomato type. Analyses of all data were done independently

for processing and fresh market tomato as two simultaneous experiments. All four rows were planted to cucumbers (*Cucumis sativas*) in sequence as described below. No disease data were collected from cucumber plots and cucumber yields are reported elsewhere (Chapter 3).

Main plots: Cropping sequence. Main plot treatments consisted of continuous tomato (no rotation) or tomato rotated with cucumber (Figure 1), a biennial cropping system commonly used by many growers in southwest Michigan. Tomato was transplanted into all plots in 1990. Tomato was tranplanted into half the plots and cucumber seeded in the other half in 1991. Immediately after the cucumber harvest (July 25), the entire 12 x 84 m main plot was plowed and drilled to a mustard crop as a rapid source of green manure and potential fungistatic and weed suppressive properties. All plots were conventionally tilled the third week of September and rye was drilled. In 1992, tomato was again transplanted into all plots.

Sub-plots: Tillage system. Two tillage systems were employed. Conventional tillage (CT) consisted of moldboard plowing to a depth of 20-23 cm when the over wintered rye was 15-20 cm high. Up to two additional field passes with a disk and/or drag was employed for field preparation prior to planting. After each tomato harvest, plots were conventionally tilled and rye was seeded.

Zone tillage (ZT) was used as a second tillage treatment and is defined as the fracturing of the soil directly below where the plant is to be established (Grajauskis 1990). In early spring (late March to early April) of each year, paraquat (Gramoxone) at the rate of 1.1 kg ha^{-1} was applied to the over wintered rye in strips 0.46 m wide on

1.5 m (row) centers. In this manner, minimal rye biomass accumulated where tomato or cucumber plants were to be established. The inter-row rye continued to grow and was desiccated with paraquat when it reached a height of 1 to 1.2 m. Over time, the-inter-row rye lodged and settled to the soil surface.

In ZT plots each spring, the Tye paratill (Tye Company, Lockney, TX) was set to fracture the soil to an approximate 35 cm depth with minor surface disturbance and no soil inversion. After the tomato harvest in 1990, rye was drilled directly into ZT plots with no additional tillage. The goal with ZT was to perform no additional tillage until the summer or fall of the second year (1991). However, soil pentrometer readings indicated sufficient soil compaction occurred over the previous year to warrant a repeated ZT procedure in the spring of 1991. Zone tillage was performed on the exact same row center where tomato plants once stood and tomato or cucumber were to be planted. All plots were conventionally tilled after harvest in 1991 as described above.

Figure 1 provides a summary of the overall 3 year crop production system employing ZT and a rotation of tomato with cucumber.

Sub-Sub plots: Fungicide treatment. Plots were not sprayed, sprayed weekly, or sprayed at intervals according to the disease forecasting model, TOMCAST. TOMCAST calculated a daily Disease Severity Value (DSV) based on the average temperature during hours when leaves were wet (Table 1) similar to the FAST model (Madden et al. 1978). Hourly mean temperature and leaf wetness were recorded using the Omnidata model DP223 temperature and leaf wetness recorder (Omnidata International, Inc., Logan, UT). Sensors were calibrated each year.

FIGURE 1: Summary of the overall 3 year crop production system employing zone tillage (ZT) and rotation of tomato with cucumber. Solid lines represent crop growth. Dotted lines represent windows of preferred time periods for agronomic inputs. Fields are conventionally tilled (CT) commencing year one and not subject to CT again until after the cucumber harvest in year two. After a mustard crop, CT is used once more and the field is planted back to tomato. Intensive two year rotations are common with some growers in SW Michigan.

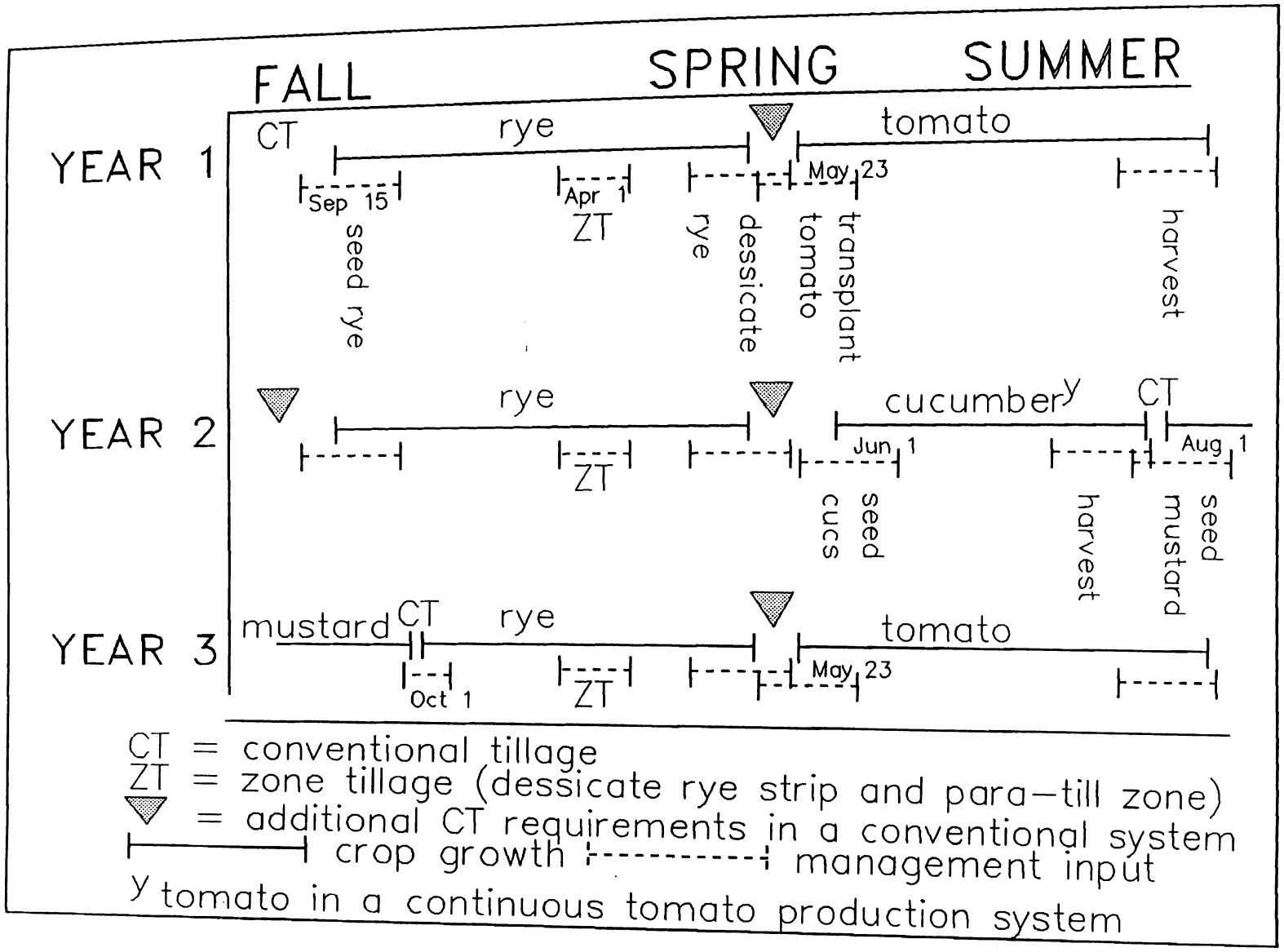

FALL
SPRING
SUMMER
YEAR 1
YEAR 2
YEAR 3
CT
rye
tomato
Sep 15
seed rye
Apr 1
ZT
rye
dessicate
May 23
tomato
transplant
harvest
rye
cucumber y
CT
Jun 1
Aug 1
ZT
cucs
seed
harvest
mustard
seed
mustard
CT
rye
tomato
Oct 1
ZT
May 23
CT = conventional tillage
ZT = zone tillage (dessicate rye strip and para-till zone)
= additional CT requirements in a conventional system
crop growth
management input
y tomato in a continuous tomato production system

TABLE 1: Number of hours of leaf wetness at a given temperature range required for each disease severity value (DSV).

Mean Temperature (C)	Disease Severity Values (DSVs)				
	0	1	2	3	4
13.0 - 17.5	0-6 hr	7-15	16-20	21+	
17.6 - 20.5	0-3	4-8	9-15	16-22	23+
20.6 - 25.5	0-2	3-5	6-12	13-20	21+
25.6 - 29.5	0-3	4-8	9-15	16-22	23+
	Hours of Leaf Wetness				

TOMCAST called for an initial spray on July 11 or earlier if DSVs reached a threshold of 35 for tomatoes planted prior to May 23 and 45 for tomatoes planted after May 23 (Pitblado 1992). Subsequent sprays were applied after the accumulation of every 15, 20 or 25 DSVs. The fungicide Bravo 720 (chlorothalonil) was used throughout the study at full recommended rate (4.2 L ha^{-1}) or at a reduced rate (2.8 L ha^{-1}). Originally, Dyrene was designed to be used prior to fruit set in one treatment followed by chlorothalonil sprays. However, initial applications coincided with fruit set and Dyrene did not need to be applied. Therefore, this treatment was sprayed according to a TOMCAST schedule (DSV 20H) in 1990 or not sprayed in 1991. Although data was collected from these plots and used to generate analysis of variance, in most cases the data is not presented since the means were no different than their corresponding duplicate treatments. Fungicide treatments, rates, and date of first application in each year are outlined in Table 2. Fungicides were applied with a hand- held boom connected with high pressure hose to an FMC tractor drawn sprayer. The boom width was adjusted with plant

TABLE 2: Date of harvest, date and rate of ethrel treatment, fungicide treatment, number of fungicide applications, and date of initial fungicide application in years 1990 to 1992.

ACTIVITY OR TREATMENT	YEAR		
	1990	1991	1992
HARVEST DATES OF FRESH MARKET TOMATO	Aug 7(218)[a]	Jul 30(210)	Aug 19(231)
	Aug 15(226)	Aug 5(216)	Aug 25(237)
	Aug 22(233)	Aug 12(223)	Sep 1(244)
	Aug 28(239)	Aug 21(232)	Sep 9(252)
	Sep 5(247)	Aug 27(238)	Sep 15(258)
	Sep 12(254)		Sep 22(265)
HARVEST DATE OF PROCESSING TOMATO	Sep 18(260)	Aug 29(240)	Sep 29(273)
DATE OF ETHREL APPLN	Sep 5	Aug 16	Sep 14
RATE OF ETHREL APPLIED	2.8 L ha^{-1}	4.2 L ha^{-1}	4.2 L ha^{-1}
FUNGICIDE TREATMENT	NUMBER OF FUNGICIDE APPLICATIONS		
WEEKLY	15(Jun 15)[b]	11 (Jun 16)	13 (Jun 25)
DSV[c] 15L[d]	NA[e]	6 (JUN 26)[b]	5 (Jul 16)[b]
DSV 15H	NA	6 (Jun 26)	4[f]
DSV 20L	4 (Jul 11)[b]	4 (Jun 26)	4 (Jul 16)
	4 (Jul 11)	4 (Jun 26)	3[f]
DSV 25L	3 (Jul 11)	NA	NA
DSV 25H	3 (Jul 11)	NA	3 (Jul 16)
NO SPRAY	0	0	0

[a] Julian Day of Year
[b] Date of intitial application for weekly or TOMCAST-based spray programs

[c] Fungicide applied after the accumulation of every 15, 20 or 25 disease severity values

[d] L = low rate of Bravo 720 (2.8 l ha^{-1}), H = high rate of Bravo 720 (4.2 L ha^{-1})

[e] Treatment not applied during this year

[f] Initial spray was inadvertantly omitted. First application = Jul 30

growth to a maximum of 1.2 m and had four swivel T-Jet nozzles, two at the boom and two as 35 cm drop nozzles, to ensure adequate coverage. The pressure was 667 kPa at the sprayer pump and a total volume of 836 L ha^{-1} was applied.

Other cultural practices. Each year nitrogen (ammonium nitrate 33-0-0) was broadcast over the rye in all plots in early April at the rate of 56 kg ha^{-1}. Cucumber and tomato received additional N at the rate of 56 kg ha^{-1} pre-plant incorporated in CT plots or banded at planting in ZT plots. An additional 28 kg ha^{-1} was sidedressed approximately 3 weeks after field setting tomato plants. An additional 56 kg ha^{-1} and 28 kg ha^{-1} was sidedressed to cucumber plots at the 3 true leaf stage and tip over, respectively. Sidedressed N was applied as a band on the soil surface beside each row. Phosphorous and potassium were applied according to recommended rates for cucumber or tomato based on soil fertility tests conducted each fall.

Trifluralin (Treflan) at the rate of 0.56 kg ha^{-1} was preplant incorporated in CT plots for control of germinating weed seeds. Post-planting weed control was achieved with cultivation in CT plots. In ZT plots, metribuzin (Sencor) was used at the rate of 0.34 kg ha^{-1}, for postemergent control of broadleaf weeds, and Fusilade was used at the rate of 0.3 kg ha^{-1}, for post-emergent control of grass weeds. Herbicide and cultivation were complimented with hand hoeing as required. Curbit was pre-plant incorporated at recommended rates for weed control in cucumber CT plots. Curbit and/or Fusilade, complemented with cultivation (CT plots only), and hand hoeing was used for postemergence weed control. Guthion was applied at recommended rates for insect control as required.

Four to five week old commercially grown tomato seedlings in 72 (FMT) or

288 (PRT) cell flats were field set the last week of May 1990 and 1991 and first 10 days of June 1992 using a conventional single row transplanter with double disk openers and a wide rubber drive. The fresh market cv. 'Pik Rite' and processing cvs. 'Ohio 7870' (1990) and 'Heinz 8704' (1991 & 1992) were spaced 0.6 and 0.3 m, respectively, on 1.5 m centers and grown by conventional ground production methods (no mulch, trellis or training). Cucumbers cv. Flurry were direct seeded early June. Overhead sprinkler irrigation was applied as needed.

Assessment of disease incidence. Percent defoliation due to early blight (necrosis and chlorosis) was assessed visually on a weekly basis after symptoms became apparent (1 to 2% severity in plots not sprayed) and continued until complete harvest of FMT fruit or treatment of PRT tomato plants with Ethrel. Assessments were based on all plants within the inner 6 m section of the inner row for each tomato type. Early season incidence of disease in 1991 and 1992 was assessed by counting the number of lesions per plant or percentage of plants with symptoms.

Tomato harvest and fruit mold incidence. Fresh market tomatoes were multiple harvested (dates shown in Table 2) from a 6 m row section when fruit reached the breaker stage or riper. All fruit were graded twice, once using market standards and again for disease symptoms. Fruit were graded for size on a commercial grader. Sizes included large fruit (No. 1) with a diameter >67 mm and medium (No. 1) fruit with a diameter of 54-67 mm. Marketable fruit with blemishes were labelled No.2's and non marketable fruit was culled. Data collected according to market standards is presented elsewhere (Chapter 3). Fruit were also sorted for symptoms of EB, ANTH, SR or

bacteria and disease incidence on fruit was expressed as a percentage of total fruit weight evaluated. Data on bacterial disease incidence is presented elsewhere (Appendix B). Processing tomatoes were treated with Ethrel and harvested by a once-over harvest (rates and dates shown in Table 2). Fruit from the 6 m harvested area was weighed and pooled. Subsequently, two subsamples collected in 20 L pails, were rated by independent teams of people according to market standards for ripe, green and cull fruit, or for incidence of ANTHR, EB or SR and expressed as a percentage of the total weight of fruit evaluated. Samples of foliage or fruit were periodically selected and pathogens isolated to verify causal organisms.

Data analysis. All data were tested for homogeneity of variance using Bartlett's test (Little and Hills 1978) before analysis of variance with Plot-IT (Scientific Programming Enterprises, Haslett, MI) or MSTAT-C (Michigan State University, E. Lansing, MI). Only in 1992 was a full three way factorial model used with fungicide as a split plot of tillage and tillage as a split plot of rotation. In 1990 and 1991 a two way factorial analysis was performed with fungicide as a split-plot of tillage using 8 (n=112) and 4 (n=56) replications, respectively.

The experiment was designed to determine the effect of rotation, tillage and fungicide treatment and their interactions. ANOVA was used to partition the degrees of freedom and associated sums of squares for the main factors and their associated interactions. With no interactions, significant effects due to rotation or tillage were determined by planned F tests calculated from the analysis of variance table using the appropriate error term. Means from significant fungicide treatment effects were separated with appropriate LSDs based on a significant F value calculated using the

overall residual mean square error of the ANOVA table (i.e. protected LSD).

Certain plots were above a field tile that malfunctioned during the experiment. Few plots were affected in 1990. Depending on the data set, 4 to 6 of 112 plots were outliers and new values were substituted using the MISVALEST subroutine of MSTAT-C. One degree of freedom for each estimated value was subtracted from the overall residual error mean square before significance of fungicide treatment effects was determined. Examples of substituted data are provided in the text. No plots were affected in 1991. In 1992, the problem persisted and impacted 2 replications of a complete treatment (i.e. numerous sub-sub-plots in CT sub-plots in rotation main plots). With unreliable data for 2 replications of complete treatments, 1992 data were analyzed over the remaining 2 replications only (n=56 rather than 112).

Mean areas under the disease progress curve (AUDPC) expressed as percent-days were calculated according to the method of Shaner and Finney (1977):

$$AUDPC=\sum_{n}^{i=1}\left[\frac{(Y_{i+1}+Y_i)}{2}\right]\left[(t_{i+1}-t_i)\right]$$

where Y_i = disease severity at the ith observation, t_i = time (days) after the initial rating at the ith observation, and n = total number of observations. Data were also transformed to assess the apparent impact of initial inoculum or rate of disease increase. The value 0.005% was added to each observation of disease incidence prior to transformation. The appropriateness of the logistic and Gompertz model was determined by comparing the coefficient of determination (R^2) and examination of scatter plots of residual terms (Campbell and Madden 1990). Selected correlation or regression analysis among data sets were performed using Pearson's correlation coefficient or a model that provided good fit, respectively.

RESULTS

Mean temperature and rainfall varied considerably over the three year study (Table 3). The first year was relatively normal, 1991 was one of the hottest summers on record, and 1992 was one of the coolest seasons on record with a wet Jul. High levels of EB, ANTH, and SR occurred each year in the absence of artificial inoculum.

TABLE 3: Mean temperature and rainfall for Southwest Michigan Research and Extension Center for 1990, 1991 and 1992.

	Temperature (C)[y]					Rainfall (mm)				
Year	May	Jun	Jul	Aug	Sep	May	Jun	Jul	Aug	Sep
1990	13.9	20.8	22.0	21.3	18.7	150	83	77	95	155
1991	19.4	23.2	23.8	22.6	17.3	41	91	80	61	81
1992	15.4	18.6	20.7	19.6	16.8	8	45	151	61	75
30 yr norm[z]	15.1	20.4	22.5	21.7	18.0	94	71	85	95	104

[y] Temperature data from O'Clare, 12 km north of Niles.
[z] 1951 - 1980

Disease progress of early blight on tomato foliage: Onset of disease varied between years. For example, at the first rating, mean incidence of defoliation due to early blight (EB) on processing tomato (PRT) plants was 1.4% (n=112), 1.8% (n=56) and 1.9% (n=56) on Aug 16 (day 227) 1990, Jul 17 (day 197) 1991, and Aug 6 (day 218), respectively (Figure 2). The highest final disease rating of PRT plots not sprayed was 25%, 55%, and 87% on Sep 5 (day 247) 1990, Aug 14 (day 225) 1991, and Sep 17

(day 260) 1992, respectively (Figure 2). The highest final disease rating for fresh market tomato (FMT) plants was 53%, 57%, and 99% on Sep 5 1990, Aug 14 1991, and Sep 17 1992, respectively (Figure 3). Final ratings of defoliation on Aug 22 (day 233) 1991 are not shown due to an epidemic of bacterial spot (*Xanthomonas campestris* pv. vesicatoria) in a number of plots that hampered the ability to rate for EB.

Variance of AUDPC values were not homogenous each year for both PRT and FMT. Therefore, AUDPC was Log_{10} transformed prior to analysis of variance and separation of means. All AUDPC values reported are back-transformed data.

Effect of reduced-sprays and tillage on AUDPC and defoliation due to EB in 1990. Tillage system and fungicide treatment significantly affected disease severity in PRT plots (Table 4, Figure 2). Zone tillage reduced mean AUDPC values 21% in PRT rows as compared to CT (Table 5). Application of chlorothlonil reduced defoliation due to early blight as compared to plots not sprayed (Table 4 and 5). Fifteen weekly applications of full rate chlorothalonil commencing June 15 did not provide superior control as compared to 4 full rate chlorothalonil sprays applied beginning July 11 and after the accumulation of every 20 DSVs (Figure 2). The main effect (averaged over CT and ZT plots, n=16) of reduced fungicide rates and applications made after the accumulation of every 25 DSVs compromised control of defoliation as compared to plots sprayed weekly (Table 5). However, final mean percent defoliation with the most liberal spray (DSV 25H) was only 12.8% (data not shown).

The interaction between tillage system and fungicide treatment was not significant (Table 4). However, reduced fungicide intergrated with ZT tillage was additive and of particular interest. For example, using an error mean square of 0.013

with 80 df and 8 observations per mean (Table 4), mean separation by LSD test of AUDPC values demonstrated all TOMCAST based application schedules combined with ZT provided control comparable to weekly sprays in CT plots (Table 5).

Four plots were affected by a broken field tile and estimated values using the MISVALEST subroutine of MSTAT-C were substituted. Estimates for pre-transformed AUDPC values were 95, 103, 186 and 177. Original values were 575, 256, 811 and 966. Weekly estimated values were also substituted to generate disease progress curves.

TABLE 4: Mean squares from analysis of variance for log area under disease progress curve (AUDPC) for foliar incidence of early blight in processing tomato (PRT) or fresh market tomato (FMT) in 1990.

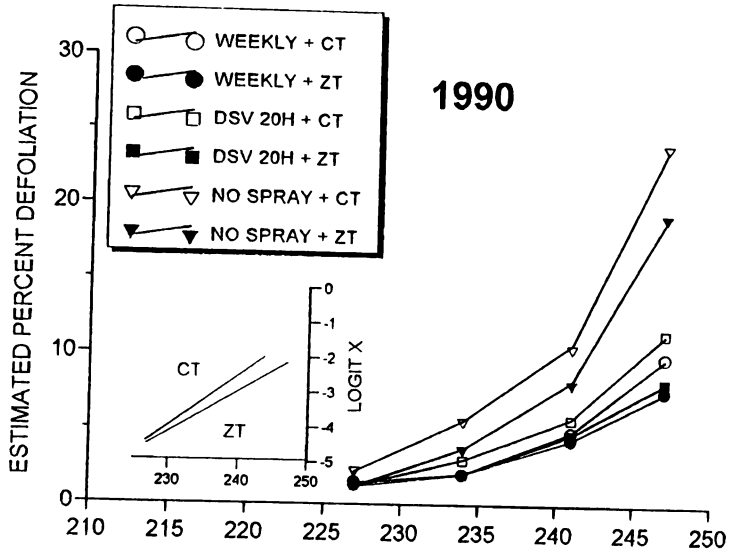
Source of Variability	df	AUDPC	
		PRT	FMT
Rep.	7[y]	0.052 NS	0.093 NS
Tillage (T)	1	0.327**	0.310*
Error a	7	0.022	0.045
Fungicide (F)	6	0.191***	0.475***
T x F	6	0.010 NS	0.031 NS
Error b	80 and 78[z]	0.013	0.0172

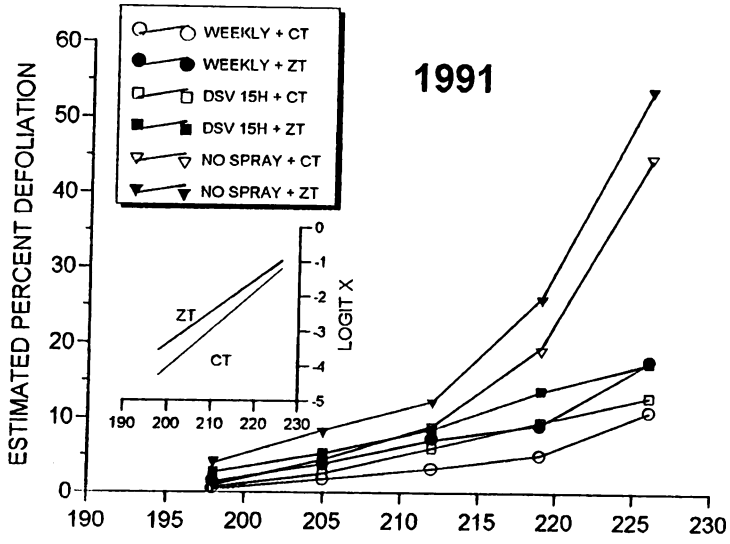
*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively. NS, non-significant.

[y] 1990 was the first year of the experiment resulting in 8 replications per treatment (i.e. different treatments were not applied to the main plots).

[z] 4 and 6 values were estimated for plots with poor drainage for processing and fresh market tomato, respectively, and 1 df for each estimated value was subtracted from the 84 df of the overall residual error mean square.

FIGURE 2: Disease progress curves of percent defoliation of processing tomato plants estimated weekly in 1990, 1991, and 1992. The insert graph of the 1990 and 1991 figures represent disease data of zone tillage (ZT) and conventional tillage (CT) plots transformed with the logistic model. Values for significance of intercept and slope are given in the text. R- = no rotation; R+ = with rotation


1990
WEEKLY + CT
WEEKLY + ZT
DSV 20H + CT
DSV 20H + ZT
NO SPRAY + CT
NO SPRAY + ZT
ESTIMATED PERCENT DEFOLIATION
LOGIT X
CT
ZT


1991
WEEKLY + CT
WEEKLY + ZT
DSV 15H + CT
DSV 15H + ZT
NO SPRAY + CT
NO SPRAY + ZT
ESTIMATED PERCENT DEFOLIATION
LOGIT X
ZT
CT

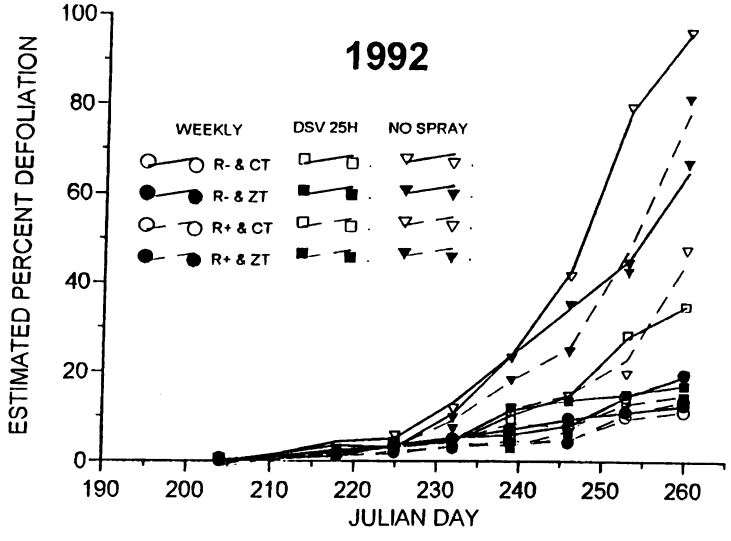
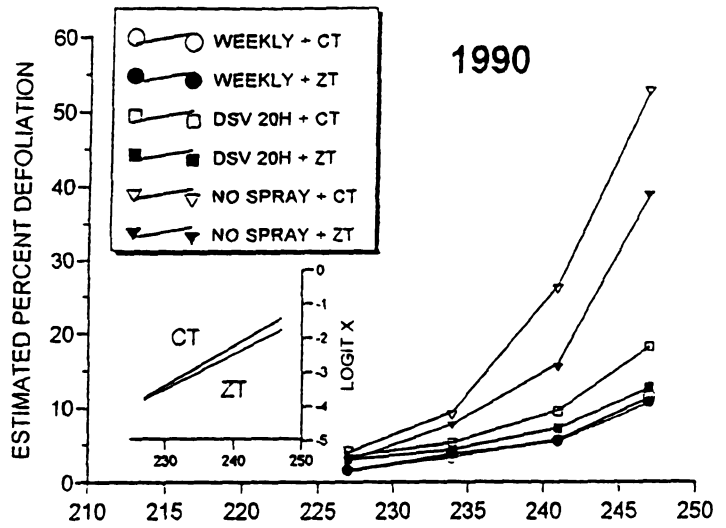
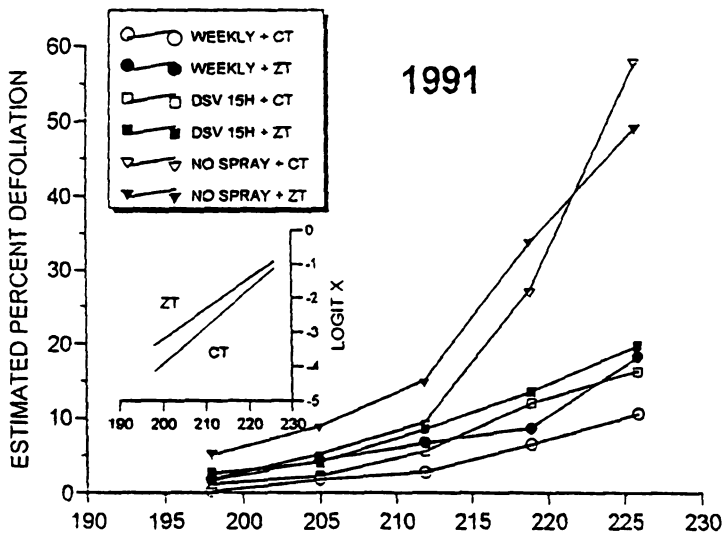

1992
WEEKLY
DSV 25H
NO SPRAY
R- & CT
R- & ZT
R+ & CT
R+ & ZT
ESTIMATED PERCENT DEFOLIATION
JULIAN DAY

FIGURE 3: Disease progress curves of percent defoliation of fresh market tomato plants estimated weekly in 1990, 1991, and 1992. The insert graph of the 1990 and 1991 figures represent disease data of zone tillage (ZT) and conventional tillage (CT) plots transformed with the logistic model. Values for significance of intercept and slope are given in the text. R- = no rotation; R+ = with rotation


1990
WEEKLY + CT
WEEKLY + ZT
DSV 20H + CT
DSV 20H + ZT
NO SPRAY + CT
NO SPRAY + ZT
ESTIMATED PERCENT DEFOLIATION
LOGIT X
CT
ZT
0
10
20
30
40
50
60
210
215
220
225
230
235
240
245
250
0
-1
-2
-3
-4
-5
230
240
250


1991
WEEKLY + CT
WEEKLY + ZT
DSV 15H + CT
DSV 15H + ZT
NO SPRAY + CT
NO SPRAY + ZT
ESTIMATED PERCENT DEFOLIATION
LOGIT X
ZT
CT
0
10
20
30
40
50
60
190
195
200
205
210
215
220
225
230
0
-1
-2
-3
-4
-5
190
200
210
220
230

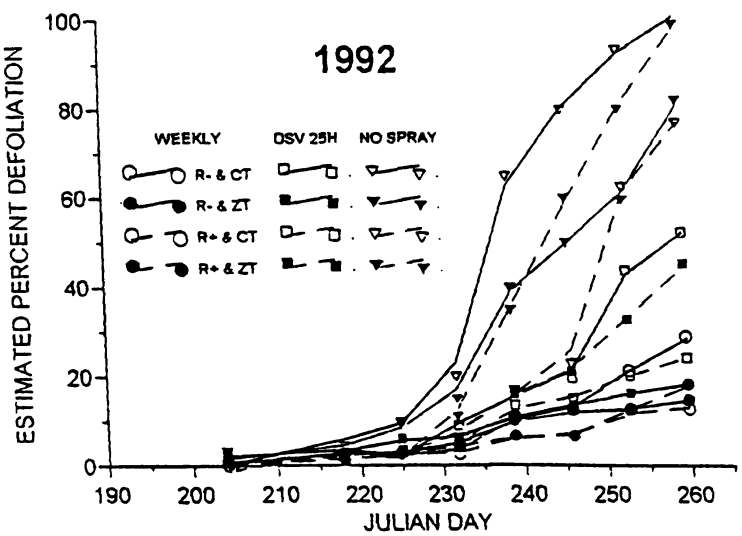

1992
WEEKLY
DSV 25H
NO SPRAY
R- & CT
R- & ZT
R+ & CT
R+ & ZT
ESTIMATED PERCENT DEFOLIATION
JULIAN DAY
0
20
40
60
80
100
190
200
210
220
230
240
250
260

TABLE 5: Backtransformed 1990 values for area under the disease progress curve for processing tomato and fresh market tomato. Analysis of variance and mean separation was based on log transformed data.

PROCESSING TOMATO			
FUNGICIDE TREATMENT	TILLAGE TREATMENT CT[p]	ZT	FUNGICIDE TREATMENT MEAN[q]
WEEKLY	74 xyz	64 z	69 d
DSV 20L	97	86 xy	91 bc
DSV 20H	95 x	71 yz	83 cd
DSV 25L	110	89 xy	100 bc
DSV 25H	118	96 x	107 b
DSV 20H	110	70 yz	90 c
NO SPRAY	180	132	156 a
MEAN OF TILLAGE	108	85	

FRESH MARKET TOMATO			
FUNGICIDE TREATMENT	TILLAGE TREATMENT CT[p]	ZT	FUNGICIDE TREATMENT MEAN[q]
WEEKLY	94 z	96 z	95 d
DSV 20L	140	121 z	130 c
DSV 20H	186	121 z	152 bc
DSV 25L	200	136	168 b
DSV 25H	149	134	142 bc
DSV 20H	141	125 z	133 c
NO SPRAY	448	263	355 a
MEAN OF TILLAGE	172	135	

[p] values are means of 8 replications. xyz is seperation of selected means within each tomato type by the Least Significant Difference (LSD) test, P = 0.05.

[q] values are means (n=16) of conventional tillage (CT) and zone tillage (ZT) plots. a-d is mean seperation within columns of each tomato type by LSD, P = 0.05.

Tillage system and fungicide treatment significantly affected disease severity in FMT plots (Table 4, Figure 3). Zone tillage reduced mean AUDPC values 22% in FMT rows as compared to CT (Table 5). Fungicide treatment reduced AUDPC values compared to plots not sprayed (Table 5). The main effect (n=16 per mean) of weekly applications was superior to each main effect of TOMCAST based treatments. However, mean separation by the LSD test of individual treatments (n=8 per mean) demonstrated reduced and full rates of chlorothalonil applied after the accumulation of every 20 DSVs and integrated with ZT offered control equal to weekly applications in CT and ZT plots (Table 5). Figure 3 highlights the disease progress curve of the DSV 20H x ZT treatment which is not significantly above disease progress curves generated from plots sprayed weekly.

Six estimated FMT AUDPC values - 123, 114, 215, 163, 431, and 480 replaced pretransformed outliers of 978, 950, 950, 768, 1352, and 1160, respectively.

In summary for 1990, ZT decreased severity of defoliation due to early blight when tomato was planted in plots with no recent history of tomato. In ZT plots, chlorothalonil applied to PRT and FMT after the accumulation of every 25 DSVs and 20 DSVs, respectively, provided control comparable to conventional production systems (weekly fungicide applications and conventional tillage). This represented 80% and 73% fewer fungicide applications without significantly compromising percent defoliation.

Effect of reduced-sprays and tillage on AUDPC and defoliation due to EB in 1991.

Tomato transplants were planted in ZT plots on the same row center as in 1990. Overwintered tomato fruit skins and dead vines were prevalent on the soil surface.

Minimal surface debris was apparent in CT plots. Commencing June 13, 11 weekly sprays of chlorothalonil were applied (Table 2). Six (DSV 15) and 4 (DSV 20) applications for the reduced-sprays program were initiated commencing June 26 after the accumulation of 35 DSVs, according to the TOMCAST model. Fruit were "walnut size" on FMT plants and just beginning to set on PRT plants on June 26.

Tillage system and fungicide treatment significantly affected disease severity in PRT plots (Table 6, Figure 2). Mean AUDPC values in PRT CT plots were 69% of mean AUDPC values in ZT plots (Table 7). Plots sprayed with chlorothalonil had significantly lower AUDPC values as compared to plots not sprayed (Table 7). Weekly fungicide applications provided superior control of defoliation as compared to all other treatment combinations (Table 7). No treatment combination provided control comparable to the conventional system, with weekly applications of fungicide and conventional tillage. Final percent defoliation recorded on Aug 14 was 14.4%, 15.3% and 16.0% for the weekly, DSV 15H and DSV 20H treatments, respectively.

Tillage system and fungicide treatment also significantly affected disease severity in FMT plots (Table 6, Figure 3). Mean AUDPC values in FMT CT plots were 69% of mean AUDPC values in ZT plots (Table 7), similar to PRT results. Plots sprayed with chlorothalonil had significantly lower AUDPC values as compared to plots not sprayed (Table7). Mean AUDPC values for main effects (n=8 per mean) of plots sprayed weekly were not significantly different than values calculated from plots sprayed with full rate chlorothalonil after the accumulation of every 15 DSVs (Table 7). Other TOMCAST-based spray treatments compromised control as compared to the weekly treatment (Table 7). However, mean separation by the LSD test of individual treatments (n=4 per mean) demonstrated reduced and full rates of chlorothalonil applied

after the accumulation of every 15 and 20 DSVs and combined with CT offered control equal to weekly applications in CT plots (Table 7).

In summary for 1991, tomato (1991) planted after tomato (1990) allowed for significant levels of defoliation in ZT plots. Conventional tillage apparently reduced initial levels of inoculum. In FMT plots, CT functioned in an additive manner when integrated with reduced fungicide applications to control defoliation equal to plots sprayed weekly. A reduced fungicide spray schedule did not provide equal control of defoliation in PRT plots as CT plots sprayed weekly.

TABLE 6: Mean squares from analysis of variance for log area under disease progress curve (AUDPC) for foliar incidence of early blight in processing tomato (PRT) or fresh market tomato (FMT) in 1991.

Source of Variability	df	AUDPC	
		PRT	FMT
Rep.	3[y]	0.062 NS	0.191 NS
Tillage (T)	1	0.372**	0.354**
Error a	3	0.011	0.010
Fungicide (F)	6	0.280***	0.289***
T x F	6	0.017 NS	0.026 NS
Error b	36[z]	0.013	0.014

*, **, *** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively. NS, non-significant.

[y] in 1991 tomato was planted to half the plots (continuous tomato main plots, n=56) and cucumber to the other half (main plots with rotation).

[z] plots with poor drainage were not located in the continuous tomato main plot treatments.

TABLE 7: Backtransformed 1991 values for area under the disease progress curve for processing tomato and fresh market tomato. Analysis of variance and mean separation was based on log transformed data.

PROCESSING TOMATO			
FUNGICIDE TREATMENT	TILLAGE TREATMENT CT[p]	ZT	FUNGICIDE TREATMENT MEAN[q]
WEEKLY	103 z	204 wxy	154 c
DSV 15L	199 wxy	306	252 b
DSV 15H	172 xy	260 w	216 b
DSV 20L	180 xy	272 w	226 b
DSV 20H	165 y	245 wx	205 b
NO SPRAY	479	469	474 a
NO SPRAY	378	516	447 a
MEAN OF TILLAGE	212	308	

FRESH MARKET TOMATO			
FUNGICIDE TREATMENT	TILLAGE TREATMENT CT[p]	ZT	FUNGICIDE TREATMENT MEAN[q]
WEEKLY	268 z	395 xy	332 c
DSV 15L	313 yz	588 w	450 b
DSV 15H	325 yz	433 z	379 bc
DSV 20L	375 yz	579 wx	477 b
DSV 20H	306 yz	632 w	469 b
NO SPRAY	929 v	927 v	928 a
NO SPRAY	940 v	1042 v	991 a
MEAN OF TILLAGE	430	621	

[p] values are means of 4 replications. v-z is seperation of selected means within each tomato type by the Least Significant Difference (LSD) test, P = 0.05.

[q] values are means (n=8) of conventional tillage (CT) and zone tillage (ZT) plots. a-c is mean seperation within each column of tomato type by LSD, P = 0.05.

Effect of reduced-sprays, tillage and rotation on AUDPC and defoliation due to EB in 1992. All plots were conventionally tilled in 1991 and the 2-year ZT cycle (Figure 1) was re-initiated in the early spring. Late season forecasted frosts delayed time of planting to Jun 3 and Jun 10 for FMT and PRT, respectively. Cool weather persisted through the spring and DSVs cumulated slowly, with average T° frequently below the 13°C threshold (Table 1) during periods when leaves were wet (e.g. night temperatures dipped to a low of 2°C June 22). The PRT experiment was terminated pre-maturely on Sep 29, 1992 to avoid forecasted frost and loss of final yield data.

Commencing June 25, 13 weekly sprays of chlorothalonil were applied (Table 2). Initial application of TOMCAST-based sprays was scheduled for the "safe date" of Jul 11 but wet weather delayed the first spray until Jul 16. Five, 4 and 3 subsequent applications were scheduled after the accum-ulation of every 15 (DSV 15L), 20 (DSV 20L) and 25 (DSV 25H) disease severity values. Plots scheduled to receive the full rate of chlorothalonil after the accumulation of every 15 (DSV 15H) or 20 (DSV 20H) DSVs were inadvertently not sprayed Jul 16. In the latter treatments, a total of 4 and 3 sprays were applied commencing Jul 30.

With only 1 df for the denominator and numerator, significant effects due to rotation were not observed (Table 8) even though mean AUDPC in rotation PRT plots was 70% of values in plots not rotated (Table 9). The main effect of CT as compared to ZT was also not significant but the rotation x tillage interaction was (Table 8). Zone tillage decreased mean AUDPC values from 696 to 479 percent-days in plots planted to continuous tomato. In contrast, mean AUDPC values increased from 368 percent-days in CT plots to 446 in ZT plots when combined with rotation (Table 9). No other interactions were significant (Table 8).

TABLE 8: Mean squares from analysis of variance for log area under disease progress curve (AUDPC) for foliar incidence of early blight in processing tomato (PRT) or fresh market tomato (FMT) in 1992.

Source of Variability	df	AUDPC	
		PRT	FMT
Rep.	1[y]	0.060 NS	0.137 NS
Rotation (R)	1	0.330 NS	0.097 NS
Error a	1	0.007	0.013
Tillage (T)	1	0.022 NS	0.006 NS
R x T	1	0.211*	0.513*
Error b	2	0.004	0.007
Fungicide (F)	6	0.204***	0.274***
R x F	6	0.002 NS	0.007 NS
T x F	6	0.005 NS	0.003 NS
R x T x F	6	0.017 NS	0.019 NS
Error c	24	0.009	0.010

*, **, *** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively. NS, non-significant.

[y] nearly all sub-sub-plot treatments in 2 replications of a sub-plot treatment (conventional tillage) of a main-plot treatment (tomato rotated to cucumber) were adversly affected by a field tile that malfunctioned, resulting in poor drainage. Therefore the data were analyzed over 2 replications instead of 4 (n=56, not 112).

TABLE 9: Backtransformed 1992 values for area under the disease progress curve for processing tomato and fresh market tomato. Analysis of variance and mean separation was based on log transformed data.

PROCESSING TOMATO					
FUNGICIDE TREATMENT	NO ROTATION CT	NO ROTATION ZT	ROTATION CT	ROTATION ZT[p]	FUNGICIDE[q] TRMT MEAN
WEEKLY	366 w-z	332 xyx	234 z	250 yz	295 c
DSV 15L	859	422 wx	320 xyz	585	546 b
DSV 15H	560	481 wx	377 wxy	402 wx	465 b
DSV 20L	794	437 wx	416 wx	448 wx	524 b
DSV 20H	614	419 wx	371 wxy	378wxy	445 b
DSV 25H	565 w	446 wx	357 xyz	351 xyz	430 b
NO SPRAY	528	1054	590	1005	1044 a
MEAN OF COLUMN	696	479	368	446	
MEAN OF ROTATION	577		405		
MEAN OF TILLAGE	CT = 506		ZT = 462		

FRESH MARKET TOMATO					
FUNGICIDE TREATMENT	NO ROTATION CT	NO ROTATION ZT	ROTATION CT	ROTATION ZT[p]	FUNGICIDE[q] TRMT MEAN
WEEKLY	516 xy	406 xyz	309 z	330 yz	390 c
DSV 15L	1483	550 x	450 xyz	1009	873 b
DSV 15H	877	679	504 xy	830	722 b
DSV 20L	1047	608 x	652 x	849	789 b
DSV 20H	857	541 x	533 x	721	663 b
DSV 25H	826	490 xyz	512 xy	741	642 b
NO SPRAY	2291	1552	1099	1722	1666 a
MEAN OF COLUMN	1019	626	542	804	
MEAN OF ROTATION	799		660		
MEAN OF TILLAGE	CT = 743		ZT = 710		

[p] values are means of 2 replications. w-z is seperation of selected means within each tomato type by the Least Significant Difference (LSD) test, P = 0.05.

[q] values are means (n=8) of conventional tillage (CT) and zone tillage (ZT) plots averaged over rotation treatment. a-c is mean seperation within each column of tomato type by LSD, P = 0.05.

Fungicide treatment significantly affected disease severity (Table 8). Plots sprayed with fungicide decreased disease severity compared to plots not sprayed and mean AUDPC values of plots sprayed weekly were significantly lower than all TOMCAST-based treatments. However, Table 9 and Figure 2 highlight the integrated effect of reduced fungicide applications when combined with rotation. The DSV 25H treatment combined with rotation provided control comparable to plots sprayed weekly in CT and ZT plots.

In the case of fresh market tomato plants, numerous plots not sprayed approached 100% defoliation. Rotation decreased mean AUDPC values 17% but this was not significant (Tables 8 & 9). Tillage also did not affect disease severity but the interaction of rotation x tillage was important (Table 8) similar to results observed for PRT. Fungicide application reduced disease severity as compared to plots not sprayed. TOMCAST-based sprays significantly controlled defoliation as compared to plots sprayed weekly (Table 9, Figure 2) but did not provide equal control as CT plots sprayed weekly and rotated to cucumbers.

Effect of fungicide and tillage on rate of disease progress. The logistic and Gompertz model both accounted for a high percentage of variation in the incidence of defoliation of tomato plants. For example, coefficients of determination (R^2) for each plot of PRT with five temporal observations in 1991 ranged from 0.88 to 0.99. Mean ($\pm$ SD) R^2 was 0.966 $\pm$ 0.027 and 0.961 $\pm$ 0.034 for the logistic and Gompertz model, respectively, and plots of residuals confirmed the acceptability of both models. The logistic model was used for all analyses in 1990 and 1991. Variance increased with means in 1992 and simple models did not provide good fit and data were not

transformed.

In 1990, the rate (r) of disease progress in PRT plots not sprayed was 0.14 logits day^{-1} as compared to 0.10 (weekly), 0.12 (DSV 20L), 0.11 (DSV 20H), 0.13 (DSV 25L), and 0.12 (DSV 25H). The rate (r) of disease progress in FMT plots not sprayed was 0.15 logits day^{-1} as compared to 0.10 (weekly), 0.10 (DSV 20L), 0.08 (DSV 20H), 0.12 (DSV 25L), and 0.11 (DSV 25H). Mean epidemic rates in CT plots as compared to ZT plots were 0.12 vs 0.11 (P=0.3) and 0.12 vs 0.10 (P=0.08) for PRT and FMT, respectively (Figures 2 & 3, insert). The level of the regression line was higher in CT compared to ZT plots in both cases (P = 0.001).

In 1991, the rate of disease progress for untreated PRT was 0.13 in plots not sprayed and 0.08, 0.11, 0.08, 0.09 and 0.09 for the weekly, DSV 15L, DSV 15H, DSV 20L and DSV 20H treatments, respectively. The rate of disease progress for untreated FMT was 0.13 as compared to 0.08 and 0.09 to 0.10 in weekly or TOMCAST-based treatments. Mean ZT curves were higher (P=0.001) for both PRT and FMT. Epidemic rates in PRT and FMT CT plots compared to ZT plots were 0.11 vs 0.10 (P=0.13) and 0.11 vs 0.09 (P=0.05), respectively (Figures 2 & 3, insert).

Early season incidence of disease. In 1991, plants in ZT plots acquired leaf lesions within 1 wk of field setting (Table 10). No conclusive isolation was obtained. Plants in ZT plots, and in the absence of rotation, also developed collar rot (Table 10), caused by *Alternaria solani*, apparently in response to high early-season day time temperatures (e.g. up to 33°C). In many cases the lesion girdled the entire stem at the soil line but early plant productivity did not appear to be affected. For example, mean PRT plant height on Jun 19 was greater (P=0.05) in ZT plots (28.6 cm) as compared to plants in

CT plots (26.5 cm). FMT plants were 39.8 and 36.2 cm (P=0.017) in ZT and CT plots, respectively.

TABLE 10. Percent plants with early season incidence (± SE) of a leaf spot and *Alternaria solani* collar rot on processing and fresh market tomato plants, 1991.

Date and Disease	Tillage Treatment	Tomato Type	
		Processing	Fresh Market
May 30 Leaf Spot	CT	5.7 ± 1.7	0.0
	ZT	7.3 ± 2.2	7.8 ± 2.2
June 6 Leaf Spot	CT	1.1 ± 0.5	7.6 ± 1.8
	ZT	50.2 ± 5.7	89 ± 3.0
June 19 Collar Rot	CT	0.1 ± 0.1	1.1 ± 0.6
	ZT	8.1 ± 1.5	40.7 ± 3.7

On Jul 16 1992, incidence (percent plants) and severity (mean no. lesions per plant ± SE) of EB lesions was assessed in weekly, DSV 15H and no spray PRT plots which had been sprayed 3, 0, and 0 times, respectively. Mean percentage of plants with lesions was 7.5 ± 1.9, 42.2 ± 9.0 and 44.4 ± 8.3% in weekly, DSV 15H and no spray plots, respectively. In CT plots, incidence was 23.3 ± 4.8% as compared to 39.4 ± 8.0% in ZT plots. The mean effect of rotation was to reduce early initial incidence from 49.0 ± 7.3% to 13.8 ± 3.3%. Incidence and severity were highly correlated and could be described by an additive exponential model (Figure 4). Lesions were small (<5 mm) and limited to 1 to 12 per plant.

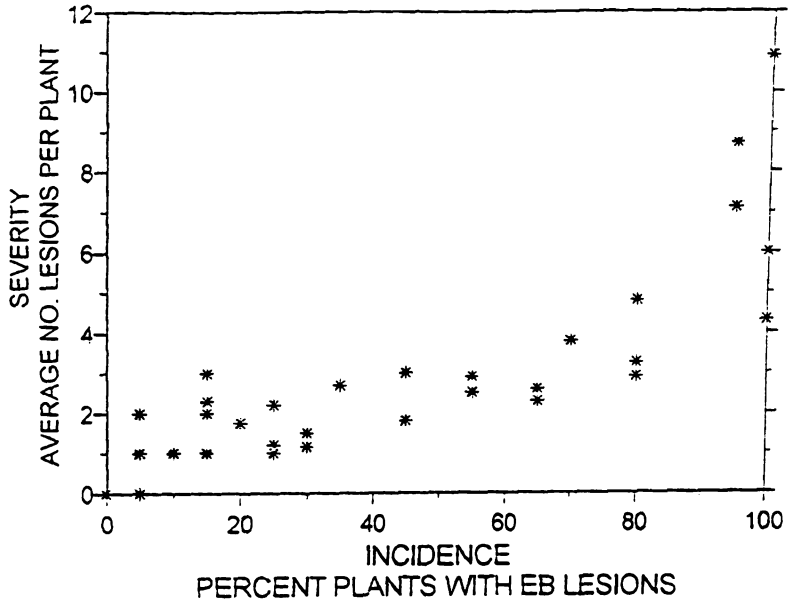


FIGURE 4:Incidence and severity of early blight symptoms recorded on processing tomato plants on July 16, 1992.

Effect of reduced-sprays, tillage and rotation on fruit mold incidence. Sub-samples of PRT fruit and all harvested FMT fruit were evaluated for symptoms of EB, ANTH, and SR without regard for severity of symptoms. The highest recorded incidence of PRT fruit mold in plots not sprayed was 20.5%, 24.0% and 52.0% in 1990, 1991, and 1992, respectively. The highest values recorded for FMT fruit mold incidence was 22.2%, 20.2%, and 19.5% in 1990, 1991, and 1992, respectively. Incidence of EB, ANTH and SR expressed as a proportion of fruit with mold symptoms was calculated for each year (TABLE 11 A & B).

Soil rot was the primary mold on FMT fruit in 1990 but the proportion steadily declined each year and EB became the primary mold problem. The proportion of each mold changed over time but was not dramatically affected by tillage nor rotation. In 1990, proportion of ANTH was 0.07 in FMT fruit harvested from ZT plots compared

TABLE 11A: Proportion of total percent fruit with mold due to early blight, anthracnose, and soil rot observed for processing tomato in 1990 to 1992.

	TREATMENT	EARLY BLIGHT			ANTHRACNOSE			SOIL ROT		
		1990	1991	1992	1990	1991	1992	1990	1991	1992
TILLAGE EFFECT	CT	0.41	0.38	0.67	0.33	0.28	0.20	0.25	0.34	0.16
	ZT	0.41	0.38	0.62	0.30	0.31	0.24	0.29	0.31	0.11
	F-VALUE	NS	NS	NS	NS	NS	NS	NS	NS	P=.004
INTERACTION										RxT
	F-VALUE									P=.001
GRAND MEAN		0.41	0.38	0.64	0.32	0.29	0.22	0.27	0.33	0.13

TABLE 11B: Proportion of total percent fruit with mold due to early blight, anthracnose, and soil rot observed for fresh market tomato in 1990 to 1992.

	TREATMENT	EARLY BLIGHT			ANTHRACNOSE			SOIL ROT		
		1990	1991	1992	1990	1991	1992	1990	1991	1992
TILLAGE EFFECT	CT	0.35	0.50	0.68	0.12	0.10	0.06	0.54	0.40	0.16
	ZT	0.29	0.37	0.55	0.07	0.12	0.07	0.65	0.5	0.26
	F-VALUE	NS	NS	NS	P=.09	NS	NS	NS	NS	NS
INTERACTION							RxT			
	F-VALUE						P=.024			
GRAND MEAN		0.32	0.44	0.62	0.09	0.11	0.07	0.59	0.45	0.21

NS = NOT SIGNIFICANT

to 0.12 in CT plots (P=0.09). Processing tomato 1992 had a higher proportion of SR in ZT plots (proportion = 0.12) as compared to CT plots (proportion = 0.07) in the absence of rotation. In contrast, proportion SR was lower in ZT plots (proportion = 0.10) as compared to CT plots (proportion = 0.25) after rotation. In 1992, proportion ANTH on PRT and FMT fruit decreased when sprayed with fungicide compared to plots not sprayed (data not shown). In contrast, proportion SR increased when fruit were sprayed weekly as compared to a reduced or no-sprays program.

Percent fruit mold incidence in 1990. Tillage did not significantly affect incidence of PRT fruit mold (Table 12). Fungicide treatment did significanlty affect PRT fruit mold incidence (Table 12, Figure 5). Full rate fungicide applications after the accumulation of every 20 (4 sprays) and 25 (3 sprays) DSVs limited mold incidence comparable to the weekly (15 sprays) treatment. The main effects (means over CT and ZT plots) are shown in Figure 5 but the integrated effect of fungicide and tillage was of particular interest. For example, ZT plots sprayed with full rate chlorothalonil after the accumulation of every 20 or 25 DSVs limited fruit mold incidence to 4.1% and 5.6%, respectively. This was not significantly different as compared to 3.5% and 3.4% in CT and ZT plots sprayed weekly. The DSV 20 and DSV 25 treatments using reduced fungicide rates compromised disease control.

Tillage did not affect incidence of fruit mold in FMT plots (Table 12, Figure 6). Fungicide treament had a significant affect on fruit mold incidence but reduced-sprays did not provide control of fruit mold in FMT plots as compared to plots not sprayed (Table 12, Figure 6). Soil rot, the predominant mold, did not appear to be effectively controlled except by weekly applications of chlorothalonil.

TABLE 12: Mean squares from analysis of variance for percent fruit with mold due to early blight, anthracnose or soil rot in processing tomato (PRT) or fresh market tomato (FMT) in 1990.

Source of Variability	df	% Fruit with Mold	
		PRT	FMT
Rep.	3[y]	258.1 NS	126.0 NS
Tillage (T)	1	280.7 NS	1.4
Error a	3	149.4	24.8
Fungicide (F)	6	140.8***	134.0***
T x F	6	13.0 NS	19.3 NS
Error b	36[z]	17.0	21.7

*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[y] fruit were harvested only from main plots destined to be in continuous tomato (n=56). Harvested PRT fruit was returned to each plot after evaluation and harvested FMT fruit was removed from all plots.

[z] plots with poor drainage were not located in the continuous main plot treatments.

Percent fruit mold incidence in 1991. Fungicide significantly affected PRT fruit mold incidence in 1991 and tillage did not (Table 13, Figure 5). Fungicide applied at the high rate and low rate after the accumulation of every 15 (6 sprays) or 20 (4 sprays) DSVs provided control comparable to the weekly (11 sprays) treatment. This represented 45% to 64% fewer fungicide applications. The tillage x fungicide interaction was not significant (Table 13). Tillage significantly affected FMT fruit mold incidence (Table 13). CT reduced FMT mold incidence as compared to ZT (Figure 6). Main effects of the DSV 15H and DSV 20H treatments were not significantly different

than the main effect of weekly applications (Figure 6). Tillage x fungicide interaction was not significant (Table 13). However, the integrated effect of reduced sprays and CT in 1991 was additive and provided control of fruit mold most comparable to plots sprayed weekly. For example the CT x DSV 15H and CT x DSV 20H treatments had a total mold incidence of 7.8% and 7.6% as compared to 5.5% and 6.8% in CT and ZT plots sprayed weekly.

TABLE 13: Mean squares from analysis of variance for percent fruit with mold due to early blight, anthracnose or soil rot in processing tomato (PRT) or fresh market tomato (FMT) in 1991.

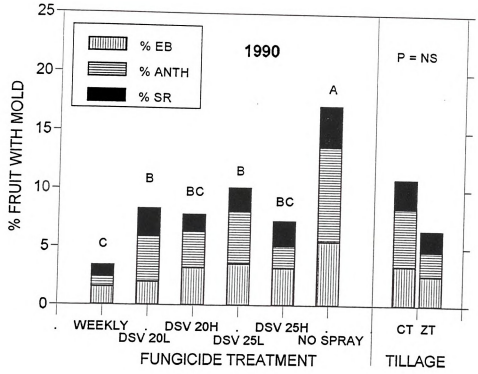
Source of Variability	df	% Fruit with Mold	
		PRT	FMT
Rep.	3[y]	95.8 NS	58.3 NS
Tillage (T)	1	236.2 NS	507.0 **
Error a	3	251.4	12.3
Fungicide (F)	6	112.4*	108.6***
T x F	6	45.0 NS	15.5 NS
Error b	36[z]	40.8	20.2

*, **, *** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non- significant.

[y] in 1991 tomato was planted to half the plots (continuous tomato main plots, n=56) and cucumber to the other half(tomato rotated to cucumber main plots).

[z] plots with poor drainage were not located in the continuous tomato main plot treatments.

FIGURE 5: Incidence of fruit mold expressed as percent of total weight of processing tomato fruit harvested in 1990 and 1991. Bars represent the main effect of each treatment (i.e. mean of CT and ZT plots combined). Bars with the same letter are not significantly different based on protected LSD value. P value indicates the level of significance between the main effect of CT as compared to ZT on incidence of fruit mold.


% EB
% ANTH
% SR
1990
P = NS
% FRUIT WITH MOLD
25
20
15
10
5
0
C
B
BC
B
BC
A
WEEKLY
DSV 20L
DSV 20H
DSV 25L
DSV 25H
NO SPRAY
CT ZT
FUNGICIDE TREATMENT
TILLAGE

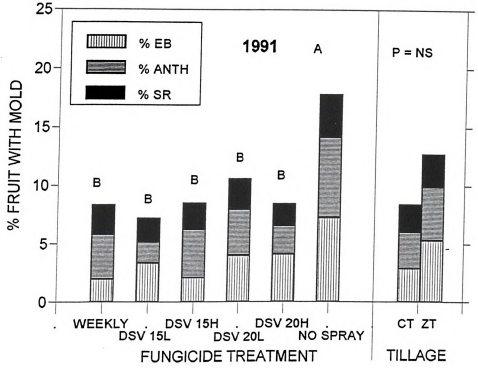
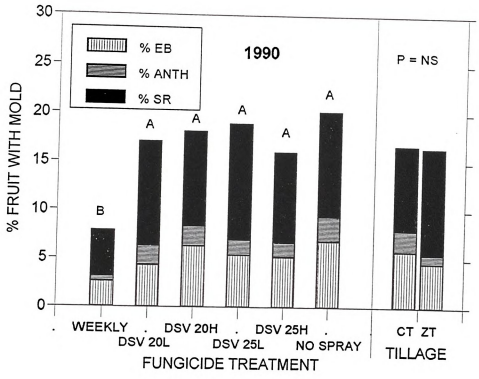
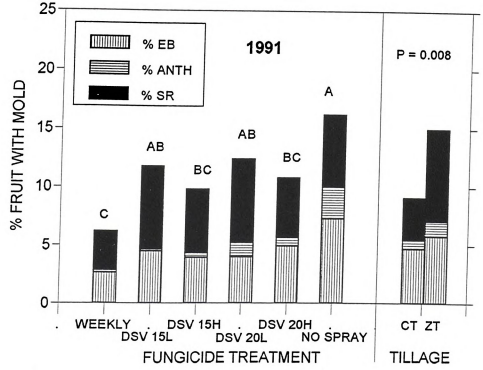

% EB
% ANTH
% SR
1991
P = NS
% FRUIT WITH MOLD
25
20
15
10
5
0
B
B
B
B
B
A
WEEKLY
DSV 15L
DSV 15H
DSV 20L
DSV 20H
NO SPRAY
CT ZT
FUNGICIDE TREATMENT
TILLAGE

FIGURE 6: Incidence of fruit mold expressed as percent of total weight of fresh market fruit harvested in 1990 and 1991. Bars represent the main effect of each treatment (i.e. mean of CT and ZT plots combined). Bars with the same letter are not significantly different based on protected LSD value. P value indicates the level of significance between the main effect of CT as compared to ZT on incidence of fruit mold.


% EB
% ANTH
% SR
1990
P = NS
% FRUIT WITH MOLD
30
25
20
15
10
5
0
B
A
A
A
A
A
WEEKLY
DSV 20L
DSV 20H
DSV 25L
DSV 25H
NO SPRAY
FUNGICIDE TREATMENT
CT ZT
TILLAGE


% EB
% ANTH
% SR
1991
P = 0.008
% FRUIT WITH MOLD
25
20
15
10
5
0
C
AB
BC
AB
BC
A
WEEKLY
DSV 15L
DSV 15H
DSV 20L
DSV 20H
NO SPRAY
FUNGICIDE TREATMENT
CT ZT
TILLAGE

Twenty fully ripe No. 1 symptomless fruit were collected from the weekly, DSV 20H, and no spray treatment on three harvest dates to determine the incidence of latent anthracnose infections and the potential market-life of harvested fruit. After one week at ambient air temperatures, over 30% of the fruit not sprayed had anthracnose lesions (Table 14). Overall, the weekly and DSV 20H treatment limited latent infections as compared to the no spray treatment. Conventional tillage significantly limited incidence of anthracnose on fruit as compared to ZT (Table 14). Effect of harvest date and interactions were not significant (data not shown).

TABLE 14. Post-harvest incidence (%) of fully red NO.1 fresh market tomato fruit with symptoms of anthracnose in 1991.

TREATMENT	HARVEST DATE			
	AUG 5	AUG 12	AUG 21	MEAN
WEEKLY	11.3 b[y]	15.4 b	24.5 a	17.1 b
DSV 20H	13.3 b	16.5 b	14.0 b	14.6 b
NO SPRAY	32.4 a	38.3 a	34.6 a	35.1 a
MEAN	19.0	23.4	24.4	
EFFECT OF TILLAGE				
CONVENTIONAL TILLAGE			19.0	
ZONE TILLAGE			25.5 (P = 0.05)	

[x] Values are means of three harvest dates with 20 fruit collected at harvest from 4 CT plots and 4 ZT plots (n=8 plots, 160 fruit).

[y] within column values followed by the same letter are not significantly different at P=0.05. Harvest date and any interaction was not significant.

[z] main effect of tillage averaged over all harvest dates and fungicide treatments.

Percent fruit mold incidence in 1992. The rotation x tillage and rotation x tillage x fungicide interactions were highly significant for both PRT and FMT in 1992 (Table 15). In general, mold incidence decreased in ZT x no-rotation plots and increased in ZT x rotation plots as compared to CT. The effect in no spray plots was most pronounced (Figure 7).

The PRT experiment was prematurely terminated and most fruit were not fully ripe. On average, fungicide applied at the full rate after the accumulation of every 25 DSVs (3 sprays) provided PRT mold control comparable to weekly (13 sprays) applications and this was influenced by cultural practice (Figure 7). For PRT plots, the DSV 25H treatment was most similar to the weekly treatment when combined with ZT or rotation (Figure 7). The DSV 15 and DSV 20 treatments with full rate chlorothalonil did not provide similar control of fruit mold as compared to plots sprayed weekly (data not shown). The initial spray for these treatments was delayed 14 days Jul 30 (Table 2) as compared to other TOMCAST-based treatments. This observation, combined with the effectiveness of the DSV 25 full rate treatment suggests that a reduced fungicide schedule is more effective than eliminating funeicide sprays early in the season.

The main effect of all TOMCAST-based treatments except the DSV 15L provided FMT fruit mold control comparable to the weekly treatment (data not shown). The DSV 25H treatment, the most liberal fungicide schedule, was equally effective in controlling FMT mold incidence for all treatment combinations as compared to plots sprayed weekly (Figure 7). Plots not sprayed had the highest overall incidence of mold (P=0.05). Figure 7 highlights the weekly, DSV 25H and no spray treatment and their interaction with rotation and tillage.

TABLE 15: Mean squares from analysis of variance for percent fruit with mold due to early blight, anthracnose or soil rot in processing tomato (PRT) or fresh market tomato (FMT) in 1992.

Source of Variability	df	AUDPC	
		PRT	FMT
Rep.	1[y]	1193.3 NS	9.8 NS
Rotation (R)	1	494.6 NS	23.8 NS
Error a	1	514.2	42.4
Tillage (T)	1	6.4 NS	>0.1 NS
R x T	1	1767.6**	113.54**
Error b	2	11.1	0.8
Fungicide (F)	6	789.8***	62.8***
R x F	6	66.9 NS	4.2 NS
T x F	6	40.6 NS	2.5 NS
R x T x F	6	225.0*	15.8*
Error c	24	69.4	5.5

*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[y] nearly all sub-sub-plot treatments in 2 replications of a sub-plot treatment (conventional tillage) of a main-plot treatment (tomato rotated to cucumber) were adversly affected by a field tile that malfunctioned, resulting in poor drainage. Therefore the data were analyzed over 2 replications instead of 4 (n=56, not 112).

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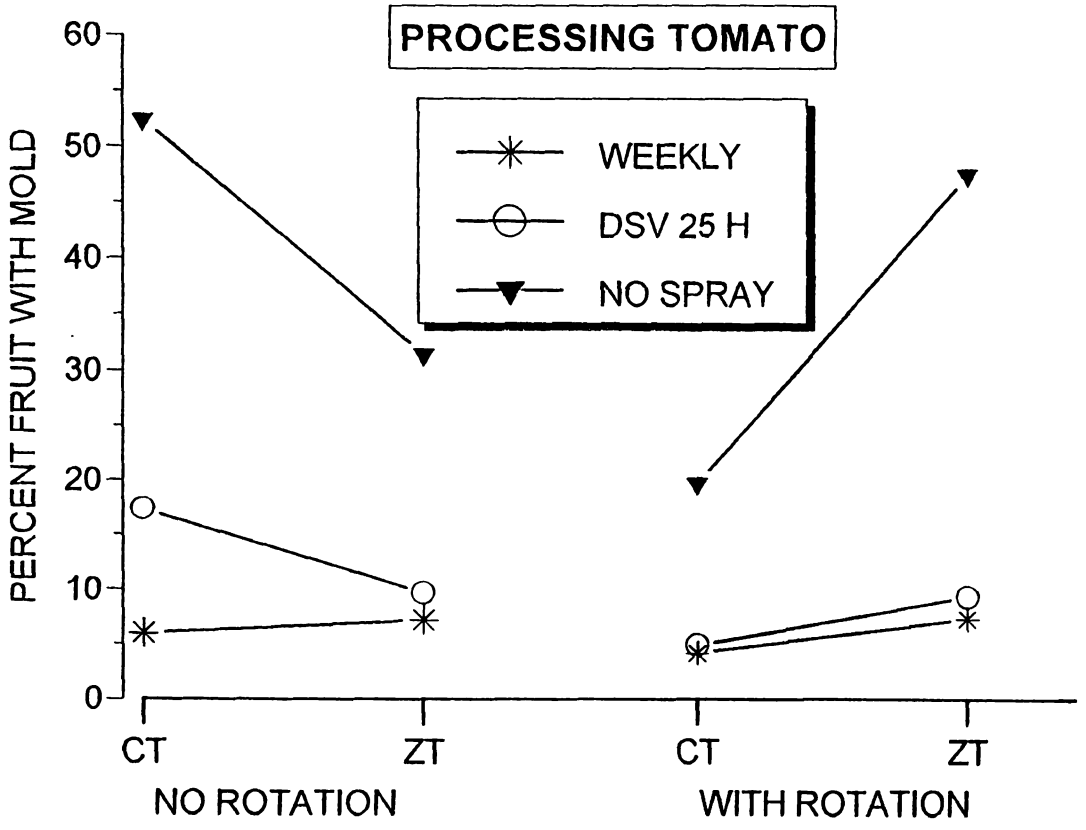
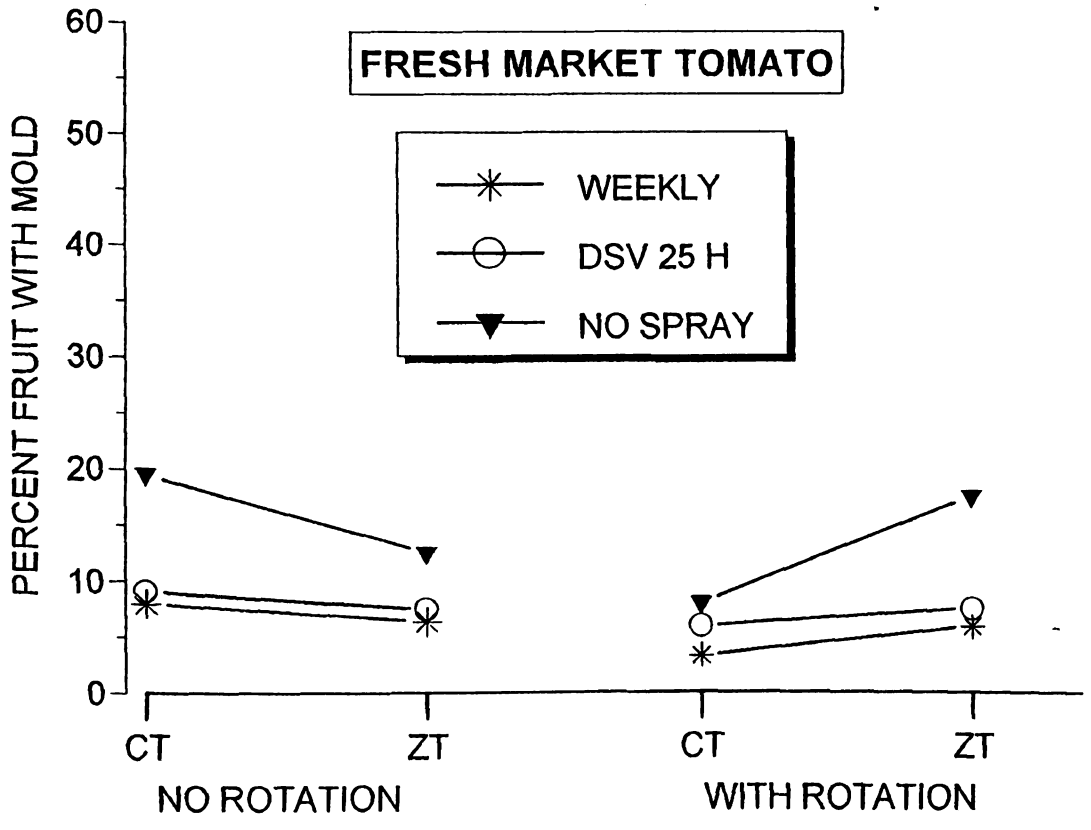
FIGURE 7: Incidence of fruit mold expressed as percent of total weight of processing (top) and fresh market (bottom) tomato fruit harvested in 1992. The 3 way interaction between rotaton, tillage and fungicide treatment was significant. Each point is the mean of 2 replications in conventional tillage (CT) or zone tillage (ZT) plots combined with a one year rotation or not rotated.

PERCENT FRUIT WITH MOLD

PERCENT FRUIT WITH MOLD


PROCESSING TOMATO
WEEKLY
DSV 25 H
NO SPRAY
PERCENT FRUIT WITH MOLD
60
50
40
30
20
10
0
CT
ZT
CT
ZT
NO ROTATION
WITH ROTATION


FRESH MARKET TOMATO
WEEKLY
DSV 25 H
NO SPRAY
PERCENT FRUIT WITH MOLD
60
50
40
30
20
10
0
CT
ZT
CT
ZT
NO ROTATION
WITH ROTATION

Effect of defoliation due to EB on total yield and incidence of fruit mold. Total yield in plots not sprayed were not significantly reduced due to defoliation for both PRT and FMT in 1990 and 1991 (Chapter 3). In 1992, lack of fungicide application did not impact FMT yield but PRT yield in plots not sprayed was reduced 25% as compared to plots sprayed with Bravo (Chapter 3, marginally significant at P = 0.06).

The incidence of fruit mold caused by EB was affected by AUDPC (non transformed values) (Figure 8). Based on analyses of data collected from 1990 to 1992, incidence of EB fruit mold increased 1.4% to 2.3% for each 100 percent-days increase in PRT AUDPC. The relationship between FMT incidence of fruit mold due to EB and AUDPC varied more among years and was not as convincing. For 1990 to 1992, the linear equation estimates varied between 0.3% to 1.2% increase in EB fruit mold with each 100 percent-days increase in AUDPC. In 1992, ANTH fruit rot increased 0.2% in FMT plots with each 100 percent-days increase in AUDPC. No relationship was obvious in 1990 and 1991, nor in PRT plots. Incidence of SR was not related to AUDPC values (data not shown).

Incidence of fruit mold due to EB and ANTH was correlated (Table 16). The correlation coefficient and slope remained relatively constant among years for FMT. The coefficient of correlation for all values over the 3 yr was 0.44 and the incidence of ANTH was 0.25 the incidence of EB. In PRT, the correlation was significant in 1990 and 1991 but not 1992. The overall correlation coefficient was not significant.

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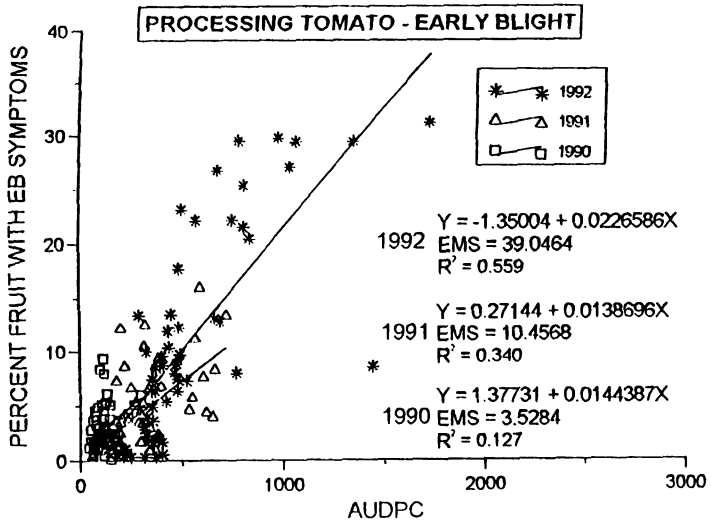
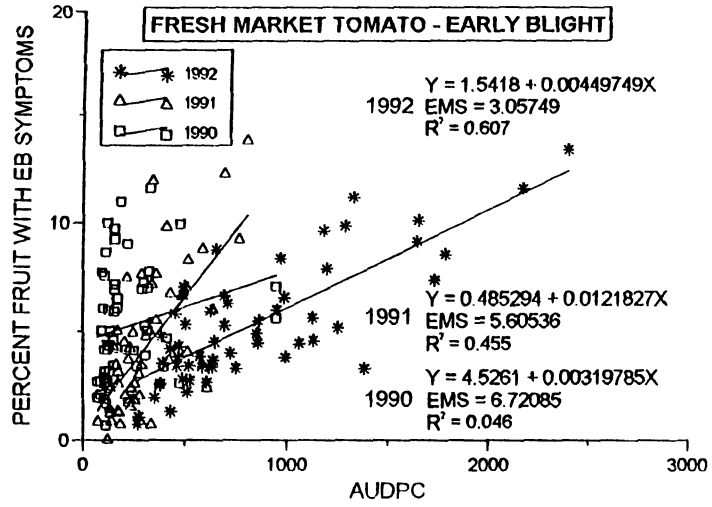
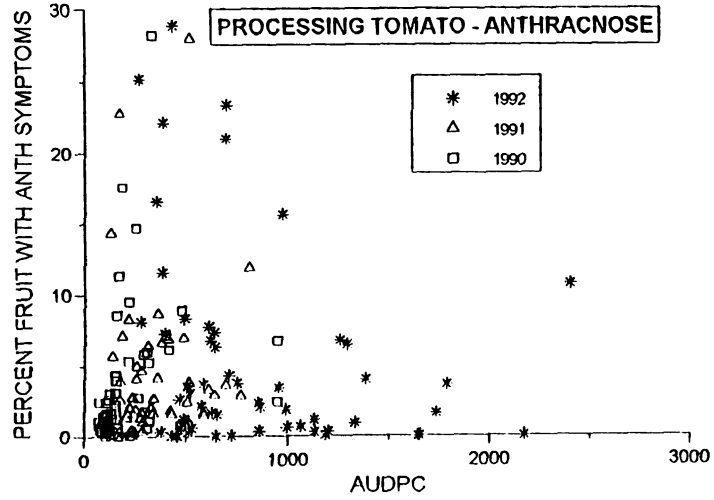
FIGURE 8. Relationship of incidence of fruit mold, due to early blight (top) or anthracnose (bottom), to non-transformed Area Under the Disease Progress Curve (AUDPC) values in processing tomato (left) and fresh market tomato (right). Equations provide the estimated intercept, slope, error mean sauare (EMS) and coefficient of determination (R^2) for linear regression analysis between early blight or anthracnose and AUDPC values.

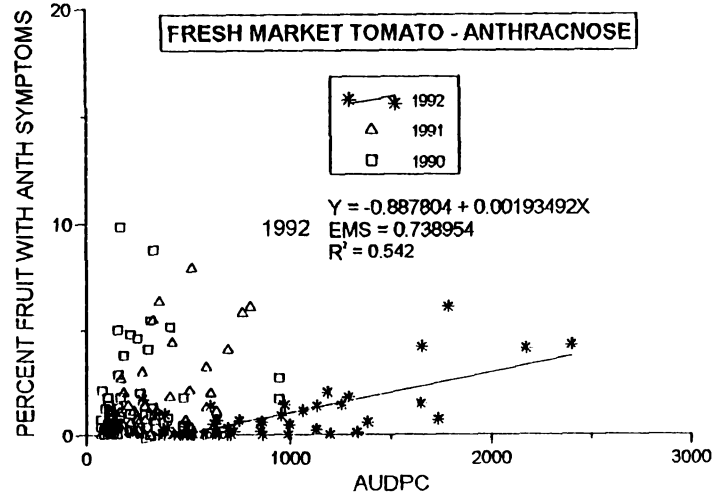

PROCESSING TOMATO - EARLY BLIGHT
40


FRESH MARKET TOMATO - EARLY BLIGHT
20
1992


PROCESSING TOMATO - EARLY BLIGHT
PERCENT FRUIT WITH EB SYMPTOMS
AUDPC
1992
1991
1990
Y = -1.35004 + 0.0226586X
1992 EMS = 39.0464
R² = 0.559
Y = 0.27144 + 0.0138696X
1991 EMS = 10.4568
R² = 0.340
Y = 1.37731 + 0.0144387X
1990 EMS = 3.5284
R² = 0.127


FRESH MARKET TOMATO - EARLY BLIGHT
PERCENT FRUIT WITH EB SYMPTOMS
AUDPC
1992
1991
1990
Y = 1.5418 + 0.00449749X
1992 EMS = 3.05749
R² = 0.607
Y = 0.485294 + 0.0121827X
1991 EMS = 5.60536
R² = 0.455
Y = 4.5261 + 0.00319785X
1990 EMS = 6.72085
R² = 0.046


PROCESSING TOMATO - ANTHRACNOSE
PERCENT FRUIT WITH ANTH SYMPTOMS
AUDPC
1992
1991
1990


FRESH MARKET TOMATO - ANTHRACNOSE
PERCENT FRUIT WITH ANTH SYMPTOMS
AUDPC
1992
1991
1990
Y = -0.887804 + 0.00193492X
1992 EMS = 0.738954
R² = 0.542

TAB
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incid
fresh

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TABLE 16: Pearson's correlation coefficient (r), intercept, slope, standard error (SE) of the slope and significance value of the correlation of the relationship between incidence of fruit mold due to early blight and anthracnose in processing (PRT) and fresh market tomato (FMT) in 1990, 1991, and 1992.

TREATMENT	r	INTERCEPT	SLOPE	SE OF SLOPE	P VALUE
1990 PRT	0.463	0.12	1.155	0.301	0.001
1991 PRT	0.402	1.61	0.543	0.177	0.003
1992 PRT					NS
1990 TO 1992 PRT	0.088	3.38	0.075	0.067	0.262
1990 FMT	0.396	-0.03	0.316	0.10	0.002
1991 FMT	0.494	0.14	0.293	0.071	0.001
1992 FMT	0.567	-0.64	0.258	0.051	0.001
1990 TO 1992 FMT	0.437	-0.11	0.275	0.044	0.001

DISCUSSION

Integrated control of early blight (EB), anthracnose (ANTH), and soil rot (SR) with reduced fungicide input was possible in a fresh market (FMT) and a processing tomato (PRT) production system. However, the dynamics of disease incidence varied with culture practice, reduced sprays, tomato type and year. Conventional tillage (CT) and weekly application of full rate fungicide required less management and appeared to minimize crop quality risk as compared to reduced-sprays and conservation tillage practices. However, conservation tillage, use of cover crops and reduced fungicide inputs have considerable benefit for soil health, soil quality, environmental quality and overall long-term sustainability of field level productivity and profitability (Coolman and Hoyt 1993; Fretz et al. 1993; Morse 1993; Sarrantonio 1992; Stephens 1990). The challenge is to combine the benefits of reduced fungicide usage without compromising the benefits of conservation tillage and cover crop use.

Zone tillage (ZT) is a form of conservation tillage designed to circumvent problems of reduced yield or plant stand associated with complete no-till production systems (Doss et al. 1981; Price and Baughan 1988). The zone tillage system employed in this study enhanced or did not affect tomato total yields when combined with rotation (Chapter 3, Grajauskis 1990). In 1990, the first year of this study, ZT significantly decreased percent defoliation due to early blight (Figure 2 & 3) as compared to a conventional tillage system. The mechanism may in part be due to the near-complete covering of the soil surface and a reduction in fungal spore dispersal. Alternatively, the primary effect may be indirect through altered host productivity and reaction to infection (Sumner et al. 1986). For example, delayed senescence is known

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to be associated with decreased susceptibility to defoliation due to EB (Horsfall and Heuberger 1942; Jones and Jones 1986; Thomas 1948). Zone tillage enhances root biomass accumulation and soil exploration (Grajauskis 1990) which could result in plants with more vigor. Visually, plants appeared greener longer throughout the season and fruit maturity was delayed (Chapter 3) as compared to CT.

Transformation of percent defoliation with the logistic model provided some understanding as to the impact of zone tillage. In 1990, the level of the curve was significantly lower in ZT plots as compared to CT plots (Figure 2 and 3). Based on the assumption that initial levels of natural inoculum were randomly distributed in ZT and CT plots prior to planting any tomatoes, ZT may have reduced the potential of initial inoculum to disperse or may have delayed senescence in host plants as described above, and the onset of an epidemic. In 1991, tomato debris from the 1990 crop persisted on the soil surface in ZT plots and functioned as a ready source of initial inoculum. The high incidence of collar rot, an uncommon disease in the northern production regions, attests to the inoculum levels that must have been present in ZT plots. Data transformed with the logistic model (inset Figure 2 and 3) illustrate the ZT curves were substantially higher than CT curves. However, in both 1990 and 1991, the mean rate of the epidemic (r) was lower in ZT plots compared to CT plots, although the differences were not always significant. The logistic data and crop productivity observations suggest ZT impacts components of the host-pathogen interaction in a functional manner similar to rate-reducing resistance (Kuhn et al. 1978), only the rate-reducing resistance is not mediated by host genotype, but by cultural impacts on phenotype. Additional work is required before the mechanism of reduced EB defoliation will be adequately known.

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The proportion of total fruit mold caused by EB, ANTH or SR was not affected by tillage in 1990 and 1991 (Table 11) even though the absolute amount of fruit with mold symptoms changed. There was a slight tendency for the incidence of ANTH to decline in ZT plots and the incidence of SR to increase, but these changes were not dramatic. These data suggest ZT uniformly impacted each component of the foliar-fruit fungal complex. This is important when devising integrated management systems. Integrated disease management systems are designed to control a complex of problems and if the proportion of each problem changes, the dynamics of disease management must also be adjusted and so, become more complex. We feared that ZT may provide a reservoir base of crop residue for the saprophytic persistence of *R. solani* (Neate 1987; Papavizas 1970) resulting in proportionally more SR problems in ZT plots compared to CT plots. This was not the case except in 1992 in continuous tomato ZT plots. Also in 1992, ZT combined with rotation had proportionally less SR than CT combined with rotation, although the total amount of disease was higher. Cultural practice also had similar effects in both FMT and PRT systems.

The reason for the higher level of disease in 1992 in ZT x rotation plots is not clear although the total weight of marketable fruit was also substantially higher (Chapter 3). Plots not sprayed appeared to be affected most dramatically by the ZT x R combination (Figure 7). In contrast, full rate fungicide applied weekly or after every 25 DSVs provided control of fruit mold comparable to the CT x rotation combination (Figure 7).

In 1991, CT proved to be important for reducing initial inoculum and, overall, reduced incidence of disease as compared to ZT plots. In the absence of rotation, CT appears to be essential to bury crop debris and reduce overwintered inoculum levels. A

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2 to 3 year rotation to non-solanaceous crops is a standard recommendation for tomato (Horsfall & Heuberger 1942, Sherf and MacNab 1986; Jones et al. 1991). Most commercial growers would not grow continuous tomato. Our purpose was to compare the impact of continuous tomato on disease level and to provide challenging levels of inoculum to test the TOMCAST model.

Applications of chlorothalonil according to the TOMCAST model after the accumulation of every 20 DSVs is considered a liberal schedule and is not as effective as a standard spray schedule in Iowa (Gleason et al. 1992). In 1990, we chose the liberal schedule of 20 and 25 DSVs as thresholds for fungicide application on the premise natural levels of inoculum would be low. In 1991, a more conservative spray program was adopted to account for predicted high levels of overwintered inoculum. In 1992, a DSV 25 threshold treatment was again included to determine the contribution rotation and tillage may offer in a reduced-sprays program.

TOM-CAST based spray schedules did not provide control of defoliation in all cases as compared to plots sprayed weekly (Figure 2 and 3, Table 5,7 and 9). However, there was no consistent relationship between AUDPC and incidence of SR or ANTH (Figure 8). No relationship was observed in PRT. In 1992, incidence of ANTH fruit mold in the FMT production system increased 0.2% for each 100 percent-days of AUDPC (non-transformed AUDPC). However, main effect mean AUDPC values of plots treated with fungicide did not exceed 1000. In 1992, 1000 percent days was approximately correlated with 60% final percent defoliation. Likewise, percent fruit with EB symptoms increased 0.3% to 2.3% for each 100 percent-days. Therefore it appears a certain level of defoliation can be tolerated before incidence of fruit mold becomes a problem.

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For example, with the exception of FMT in 1990, selective TOM-CAST scheduled chlorothalonil applications reduced percent fruit with mold comparable to a weekly application schedule (Figure 5 to 7). In 1991 and 1992 in the case of FMT, and in 1990, 1991, and 1992 in the case of PRT, the most liberal TOMCAST-based, full-rate spray schedule provided control of fruit mold when combined with ZT, CT and rotation in 1990, 1991, and 1992, respectively. This represented 64% to 80% fewer fungicide applications.

Application of reduced fungicide rate (2.8 L ha^{-1}) introduced more risk in terms of decreased fruit quality, as compared to full rate (4.2 L ha^{-1}) treatments, even if applications were more frequent. Therefore, reduced total fungicide input appears to be managed best by proper scheduling of fewer sprays as opposed to reduced fungicide rates at a more frequent schedule. Weekly fungicide sprays, as compared to the no spray treatment, tended to decrease the proportion of fruit mold caused by *C. coccodes* and this was complemented by an increase in the proportion of SR, caused by *R. solani*. These data suggest chlorothalonil is not equally effective in the control of ATHR and SR. McCarter and Barksdale (1977) noted fungicide applications at regular intervals during fruit development can be moderately effective in providing control of SR but this is not considered a reliable, economic approach (Jones et al. 1991).

C.coccodes is able to colonize and sporulate on senescent foliage (Farmer 1959; Illman et al 1959; Pantidou and Schoeder 1955; Younkin et al. 1944) and this is believed to act as a "bridge" between initial inoculum from the soil and secondary inoculum that affects fruit. In this study, the poor relationship between AUDPC and incidence of ANTH fruit rot suggests this is not the mechanism by which inoculum levels of *C. coccodes* build up. Alternatively, defoliation may affect the

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microenvironment or fruit susceptibility and favor ANTH fruit rot (Sherf and MacNab 1986). Considerable research is required to determine the relationship between initial sources of inoculum, the relative importance of foliar infection, the potential of secondary buildup of inoculum on leaf tissue, and the inoculum responsible for fruit infection.

The correlation between incidence of EB and ANTH, with the exception of 1990 PRT data (Table 16), suggests conditions that favor EB also favor ANTH. This may account for the effectiveness of the TOMCAST model to provide control of both fruit molds. There was no consistent correlation between AUDPC value, percent EB or percent ANTH with SR. This was foreseen since the biology and epidemiology of *R. solani* differs considerably as compared to *A. solani* and *C. coccodes*. *R.solani* is a soil borne pathogen and does not produce spores. The pathogen is a serious concern with tomato fruit in contact with soil (Jones and McCarter 1974).

Genetic and agronomic production differences between fresh market (FMT) and processing tomato (PRT) production systems also impacted the level and proportion of early blight (EB), anthracnose (ANTH), and soil rot (SR). FMT plants were planted at half the density as compared to PRT plants and bore a heavier early and total fruit load. Earliness and fruit load are associated with increased susceptibility to *A. solani* (Barratt and Richards 1944; Horsfall and Heuberger 1942; Sherf and MacNab 1986) as opposed to a true resistance mechanism. Maximum percent defoliation of FMT plants was two-fold greater in 1990 as compared to PRT plants and continued to be higher throughout the study, even with a PRT cultivar change in 1991. Presumably, inoculum levels associated with overwintered debris, volunteer plants, or alternative hosts increased from one year to the next leading to progressively higher levels of defoliation

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each year. However, the dramatic increase in percent defoliation in 1992 was influenced by late harvest dates and a prolonged period for epidemics to develop.

Fresh market tomato are thin skinned compared to processing tomato cultivars. This may account for the high incidence of SR on FMT fruit as compared to PRT fruit in 1990. *R. solani* is influenced more by the previous crop than by tillage system (Sumner et al. 1986; Rush and Winter 1990) although there is considerable variability in the biology and ecology among anastomosis groups. Hayslip and Stall (1959) demonstrated SR is much more severe when a grass plus a white clover cover crop precedes tomato than when grass alone precedes tomato. Rush and Winter (1990) reported sugar beets following alfalfa had the highest incidence of disease as compared to sugar beets following cotton, fallow or sunflower. Brunson et al. (1993) noted populations of *R. solani* in a cantaloupe production system were significantly higher in fields previously planted to crimson clover and subterranean clover cover crops. In our study, a rye crop preceded the tomato crop. Limited information is available concerning the impact of tillage and previous crop on incidence of soil rot in tomato fruit.

FMT and PRT production systems differ in the mode of harvest. FMT fruit are picked in multiple harvests at the breaker stage or riper, and marketed. Harvest of ripe PRT fruit is often delayed for extended periods to allow optimum timing for once-over machine harvesting. Because ANTH symptoms appear only as fruit ripen (Fulton 1948), incidence of ANTH at harvest can be high in PRT production systems (Precheur 1992) but not FMT systems. In our study, ANTH comprised approximately 22 - 32% of total PRT fruit mold as compared to 7.0 - 11% of total FMT fruit mold (Figure 5 and 6). However, ANTH can be a post-harvest problem if consumption is delayed. Table 14 shows that TOMCAST based sprays can be equally effective as weekly

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Finally, because FMT fruit was removed from vines weekly, there is less of a relationship between the AUDPC values and incidence of EB fruit mold (Figure 8) as compared to PRT. Although *A. solani* can infect fruit directly, fruit invasion occurs primarily through the stem end (Horsfall and Heuberger 1942) apparently via the calyx or pedicle. Premature removal of fruit likely limits migration of the pathogen from stem tissue into the fruit.

In this study, in addition to the affect on disease levels, substantial benefit in terms of reduced wind and water soil erosion, wind damage to plants, reduced early season Colorado potato beetle populations, and yield were achieved with ZT (Chapter 3, Appendix B). This cultural practice holds considerable promise for vegetable-based production systems. A reduced-sprays program was effectively incorporated into the production system and was successful, in part, due to the integrative impacts of cultural and chemical based control strategies. However, a higher level of management was required when both strategies were combined. This combination makes the production system more dependent on an enhanced knowledge base as opposed to production inputs (Fretz et al. 1993). This will prove to be a challenge for the farm manager. Ultimately, integrated management of indigenous diseases (i.e. diseases that originate and persist at the farm level) such as EB, ANTH and SR is dependent on the ability of the farm manager to integrate available knowledge into a working production system. However, private and public research and extension must provide a framework and knowledge resource base from which the farm manager can draw. Currently, in tomato production systems, the knowledge base lags behind the stimulus to change production systems.

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CHAPTER III

CONSERVATION TILLAGE AND ROTATION TO CUCUMBER IN TOMATO PRODUCTION SYSTEMS.

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ABSTRACT

Zone tillage (ZT) was compared to conventional tillage (CT) in a fresh market (FMT) and processing tomato (PRT) production system that included rotation to cucumber, use of rye cover crops, a mustard green manure, and reduced fungicide input. A multi-factorial experimental design was used from 1990-1992. For ZT, after fall-seeded rye was chemically desiccated in Apr in strips 0.46 m wide on 1.5-m centers, the soil was fractured to a depth of 35 cm with minor surface disturbance and no soil inversion. Remaining inter-row rye was desiccated in May and persisted throughout the summer providing up to 90% or more cover of the soil surface. CT utilized spring mold board plowing and discing. Soil surface residue in CT plots was less than 4%. ZT enhanced PRT yield in 1990, did not affect FMT marketable NO.1 fruit and weight of cull fruit and decreased weight of FMT No.2 fruit. After harvest, rye was seeded followed by CT and ZT in the spring and cucumber (pickling type) or tomato was planted. Cucumber yield from a once-over harvest (Jul 25) was not significantly affected by tillage. After the cucumber harvest, CT was performed and plots were seeded to a mustard crop as a late summer fallow. All plots were conventionally tilled mid-Sep, seeded to rye, followed by ZT or CT in the spring (1992) and planted to tomato. Rotation did not affect FMT nor PRT yield in 1992. ZT enhanced total yield of PRT (P=0.05) and did not impact yield of FMT. Forecast (TOMCAST) generated chlorothalonil spray schedules did not compromise marketable yield in the FMT or PRT production system during the 3 yr study but required 45% to 80% fewer sprays as compared to a standard weekly spray program.

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INTRODUCTION

Conservation tillage and cover cropping systems have not been adopted with equal success in vegetable production systems (Kelly 1990; Sarrantonio 1992) as compared to field crop production systems (Gebhardt et al. 1985; Phillips et al. 1980). Vegetable production systems often have a high value land base with multiple cropping of high value crops, using intensive tillage and fertilizer inputs (Kelly 1990). Specialized production systems, often with substantial investments in mechanized overhead costs, impact a growers ability or desire to include alternative cropping sequences that would enhance or build up soils. For example, few vegetable enterprises are able to include long rotations to a legume cover crop (Sarrantonio 1992) despite proven benefits toward the long term sustainability and productivity of the soils (Frye and Blevins 1989). Vegetable crops are also directly consumed by people and demand for quality is high, directly impacting the perceived and real importance of pesticide inputs. Considerable research is required to make vegetable production systems more sustainable and less input intense.

Advancements in reduced tillage and cover crop use have recently been made in numerous vegetable production systems including tomato (Abdul-Baki and Teasdale 1994; Doss et al. 1981; Knavel et al. 1977; McKeown et al. 1988; Price and Baughan 1988; Shelby et al. 1988). The purpose of this study was to determine the impact on marketable yield of fresh market and processing tomato using zone tillage within a biennial crop cycle (tomato/cucumber) that included rye cover crops and a mustard green manure. Zone tillage (ZT) is defined as the fracturing of the soil below where plants are to grow (Grajauskis 1990).

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MATERIALS AND METHODS

Location and design of field experiments. Location, design and cultural practices have been described previously (Chapter 2). Assessments of plant phenology and growth, and determination of percent surface rye residue was done in each sub-sub-plot of 3 fungicide treatments. The 3 sub-sub-plots were plots sprayed weekly, plots not sprayed and plots sprayed with full rate chlorothalonil after the accumulation of every 20 (1990) or 15 (1991, 1992) disease severity values.

Assessment of surface rye residue. Percent rye residue coverage of the soil surface was estimated according to the method of Slonneker and Moldenhauer (1977) at monthly intervals in 1991 and 1992. A 1.3 cm diameter nylon rope with 50 knots 15.2 cm apart was laid lengthwise in plots or diagonally (i.e. not parallel to tillage operation) across 3 plot rows and 3 alleys, inclusive. Percent surface residue was determined by the number of knots that touched residue divided by the total number of knots multiplied by 100.

Assessment of plant phenology and growth. A 6 m row length from which all data were obtained (as described previously in Chapter 2), was delineated by flags early in each season. Plant height and phenology data in 1991 and 1992 were recorded for the second, fifth and eighth plant from the south flag in FMT rows, and the fifth, tenth and fifteenth plant from the south flag in PRT rows. Data were analyzed on the averages of 3 plants per plot per tomato type. Plant phenology and growth were not recorded in 1990.

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Tomato harvest and evaluation. Fresh market tomato (FMT) cv 'Pik -Rite' were multiple harvested (dates shown in Table 1) from a 6 m row section when fruit reached the breaker stage or riper. All fruit were graded twice, once using market standards and again for disease symptoms. Fruit were graded for size on a commercial grader. Sizes included large fruit (No. 1) with a diameter >67 mm and medium (No. 1) fruit with a diameter of 54-67 mm. Marketable fruit with blemishes were labelled No.2's and non marketable fruit was culled. Fruit were also sorted for symptoms of diseases and reported elsewhere (Chapter 2, Appendix B).

Processing tomato (PRT) cv. OHIO 7870 (1990) and HEINZ 8704 (1991 & 1992) were treated with Ethrel and harvested by a once-over harvest (rates and dates shown in Table 1). Fruit from the 6 m harvested area was weighed and pooled. Subsequently, two subsamples collected in 20 L pails were rated by independent teams of people according to market standards for ripe, green and cull fruit or for incidence of anthracnose, early blight and soil rot. Data on incidence of disease are reported elsewhere (Chapter 2).

Cucumber harvest and evaluation: Cucumber cv. Flurry (pickling type) were once-over harvested Jul 25 and graded on a commercial grader for size and subsequently sorted for quality. Grade size and quality classes are defined in Table 7.

TABLE 1: Date of ha
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TABLE 1: Date of harvest, date and rate of ethrel treatment, fungicide treatment, number of fungicide applications, and date of initial fungicide application in years 1990 to 1992.

ACTIVITY OR TREATMENT	YEAR		
	1990	1991	1992
HARVEST DATES OF FRESH MARKET TOMATO	Aug 7(218)[a]	Jul 30(210)	Aug 19(231)
	Aug 15(226)	Aug 5(216)	Aug 25(237)
	Aug 22(233)	Aug 12(223)	Sep 1(244)
	Aug 28(239)	Aug 21(232)	Sep 9(252)
	Sep 5(247)	Aug 27(238)	Sep 15(258)
	Sep 12(254)		Sep 22(265)
HARVEST DATE OF PROCESSING TOMATO	Sep 18(260)	Aug 29(240)	Sep 29(273)
DATE OF ETHREL APPLN	Sep 5	Aug 16	Sep 14
RATE OF ETHREL APPLIED	2.8 L ha^{-1}	4.2 L ha^{-1}	4.2 L ha^{-1}
FUNGICIDE TREATMENT	NUMBER OF FUNGICIDE APPLICATIONS		
WEEKLY	15(Jun 15)[b]	11 (Jun 16)	13 (Jun 25)
DSV[c] 15L[d]	NA[e]	6 (JUN 26)[b]	5 (Jul 16)[b]
DSV 15H	NA	6 (Jun 26)	4[f]
DSV 20L	4 (Jul 11)[b]	4 (Jun 26)	4 (Jul 16)
	4 (Jul 11)	4 (Jun 26)	3[f]
DSV 25L	3 (Jul 11)	NA	NA
DSV 25H	3 (Jul 11)	NA	3 (Jul 16)
NO SPRAY	0	0	0

[a] Julian Day of Year

[b] Date of intitial application for weekly or TOMCAST-based spray programs

[c] Fungicide applied after the accumulation of every 15, 20 or 25 disease severity values

[d] L = low rate of Bravo 720 (2.8 l ha^{-1}), H = high rate of Bravo 720 (4.2 L ha^{-1})

[e] Treatment not applied during this year

[f] Initial spray was inadvertantly omitted. First application = Jul 30

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Data analysis. All data had homogenous variance as determined by Bartlett's test (Little and Hills 1978) and analysis of variance was performed with Plot-IT (Scientific Programming Enterprises, Haslett, MI) or MSTAT-C (Michigan State University, E. Lansing, MI). Only in 1992 was a full three way factorial model used with fungicide as a split plot of tillage and tillage as a split plot of rotation. In 1990 and 1991 a two way factorial analysis was performed with fungicide treatment as a split-plot of tillage using 8 (n=112) and 4 (n=56) replications, respectively. The experiment was designed to determine the effect of rotation, tillage or fungicide treatment and their interactions. ANOVA was used to partition the degrees of freedom and associated sums of squares for the main factors and their associated interactions. If the interactive effects were not significant, significant effects due to rotation or tillage (main effects) were determined by planned F tests calculated from the analysis of variance table using the appropriate error term. Means from significant fungicide treatment effects were separated with appropriate LSDs based on a significant F value, calculated using the overall residual mean square error of the ANOVA table (i.e. protected LSD). Certain plots were above a field tile that malfunctioned during the experiment. No values were obvious outliers in 1990. In 1991, values for 4 cucumber plots were estimated using the MISVALEST subroutine of MSTAT-C. No tomato plots were affected in 1991. In 1992, the problem persisted and impacted 2 replications of a complete treatment (i.e. many sub-sub-plots in CT sub-plots in rotation main plots). With unreliable data for 2 replications of complete treatments, 1992 data were analyzed over the remaining 2 replications only (n=56 rather than 112).

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TABLE 2: Percent
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RESULTS

The ZT system required a higher level of management than the CT system. Management decisions included timing of herbicide application for preparation of strips and final desiccation of rye, timing of zone tillage and precision timing of post-emergent herbicides. However, multiple advantages associated with the ZT production system, and not directly related to plant productivity, were observed.

Desiccated rye in ZT plots progressively lodged and persisted throughout the season. Data for 1992, similar to data of 1991 (not shown), is presented in Table 2. Surface residue in CT plots was 3.3% or less as compared to greater than 90% in ZT plots. Percent residue measured across the plot in ZT plots was lower than percent residue measured lengthwise between 2 rows. Early in the season, row centers had a low level of residue due to the strip application of herbicide and zone tillage. Later in the season, this area was covered by tomato foliage and not assessed.

TABLE 2: Percent surface rye residue (± standard error) in plots conventionally tilled (CT) or managed by a zone tillage (ZT) system in 1992.

TILLAGE	JUN 25		JUL 23		SEP 3
	CW[1]	LW[1]	CW	LW	LW
CT	2.5 ± 0.5	3.3 ± 0.6	2.9 ± 0.6	1.1 ± 0.3	1.6 ± 0.5
ZT	90.0 ± 0.9	94.4 ± 4.2	90.4 ± 1.0	96.9 ± 1.2	93.6 ± 1.9

[1] Percent residue was determined cross-wise (CW) across 3 row centers and alleys internal to each plot.

[2] Percent residue was determined length-wise (LW) between two rows within each plot.

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Soil maintenance qualities of the rye residue was qualitatively observed after several rain storms over the 3 year study. Soil erosion was obvious in CT plots as compared to ZT plots. The potential of reducing wind erosion of soil and protecting plants from excessive winds was observed on Jul 2, 1992, 3 weeks after field setting the tomato plants. A severe sand storm occurred and immediately after the storm, up to 100% of tomato plants were noted to be wind wiped and tilted toward the soil surface in CT plots. Plants in ZT plots appeared to remain upright. ZT allowed for the persistence of surface rye residue to limit soil and wind erosion, and wind-wiping damage to plants.

1990 SUMMER SEASON - Tomato fruit yield and quality: Analysis of variance revealed that tillage did not affect yield of No. 1 large fruit, No.1 medium fruit, nor culls of fresh market tomato but weight of No.2 fruit was less in ZT plots (Table 3, 4A). Total yields were not affected by tillage but ZT appeared to delay maturity based on the total yields obtained on each harvest date (Figure 1).

Fungicide affected yield and fruit quality (Table 3). Yield of marketable No.1 fruit in plots treated with chlorothalonil after the accumulation of every 20 or 25 DSVs was similar to plots sprayed weekly (Table 4B). Percent No.1 fruit was similar in the weekly, DSV 20L, and DSV 25H treatments. Yield and percent of No.1 fruit was compromised in plots not sprayed as compared to plots sprayed weekly. With regard to weight of cull fruit, the interaction of fungicide x tillage was significant (Table 3). Weight of culls decreased in the no spray, DSV 20L, DSV 20H, DSV 25L and DSV 25H treatments, and increased in DSV 20H and weekly treatments in ZT as compared to CT plots (Table 4C). CT plots sprayed weekly had the lowest weight of culls. Total yield was not affected by fungicide treatment (Figure 2).

TABLE 3: Mean s

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TABLE 3: Mean squares from analysis of variance for yield and fruit quality of fresh market tomato (FMT) in 1990.

Source of Variability	df	FMT FRUIT QUALITY AND YIELD (MT/HA)[x]				
		LARGE #1	%LRG #1	MED #1	# 2	CULL
Rep.	7[y]	136 NS	367 **	60 *	464 **	123 NS
Tillage (T)	1	528 NS	138 NS	60 NS	472 *	17 NS
Error a	7	124	62	14	50	77
Fung. (F)	6	143 *	102 **	18 NS	45 NS	68 *
T x F	6	35 NS	48 NS	6 NS	20 NS	87 **
Error b	84	58	27	11	41	26

*, **, *** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for large #1, % large #1, medium #1, #2 and culls.

[y] 1990 was the first year of the experiment resulting in 8 replications per treatment (i.e. different treatments were not applied to the main rotation plots).

TABLE 4A. Effect of tillage on marketable yield (metric tonnes) of fresh market tomato in 1990.

TILLAGE	LARGE NO.1		MT/Ha		
	MT/Ha	%	MEDIUM NO.1	NO.2	CULL
CT	33.6	39	9.9	23.9	19.3
ZT	29.3	37	11.4	19.8	186
P VALUE	0.08	0.18	0.08	0.02	0.6

TABLE 4B. Effect

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TABLE 4B. Effect of fungicide treatment on marketable yield (metric tonnes) of fresh market tomato in 1990.

FUNGICIDE TREATMENT	LARGE NO.1		MT/Ha		
	MT/Ha	%	MEDIUM	NO.2	CULL
WEEKLY	34.5 A[x]	42 A	10.8	21.2	15.3
DSV 20L	34.5 A	39 AB	11.3	23.5	21.2
DSV 20H	30.9 AB	38 B	11.2	21.4	18.3
DSV 25L	32.5 A	37 BC	10.4	24.0	21.4
DSV 25H	28.8 AB	39 AB	8.4	19.0	18.9
NO SPRAY	26.3 B	34 C	10.9	21.5	19.3
P VALUE	0.03	0.002	0.14	0.37	[y]

[x] means followed by the same letter within a column are not significantly different based on LSD (P=0.05, n=8).

[y] interaction significant (P=0.01).

TABLE 4C. Means of cull weight of fungicide x tillage interaction of fresh market tomato in 1990.

FUNGICIDE TREATMENT	CULLS MT/Ha	
	CT	ZT
WEEKLY	10.5 C[x]	20.0 AB
DSV 20L	23.3 AB	19.0 AB
DSV 20H	19.8 AB	16.9 B
DSV 25L	22.8 AB	19.9 AB
DSV 25H	19.6 AB	18.1 AB
NO SPRAY	20.5 AB	18.1 AB

[x] means followed by the same letter across both columns are not significantly different based on LSD (P=0.05, n=8).

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FIGURE 1: Effect of tillage and rotation on fresh market cv. 'Pik-Rite' tomato yields harvested each week.

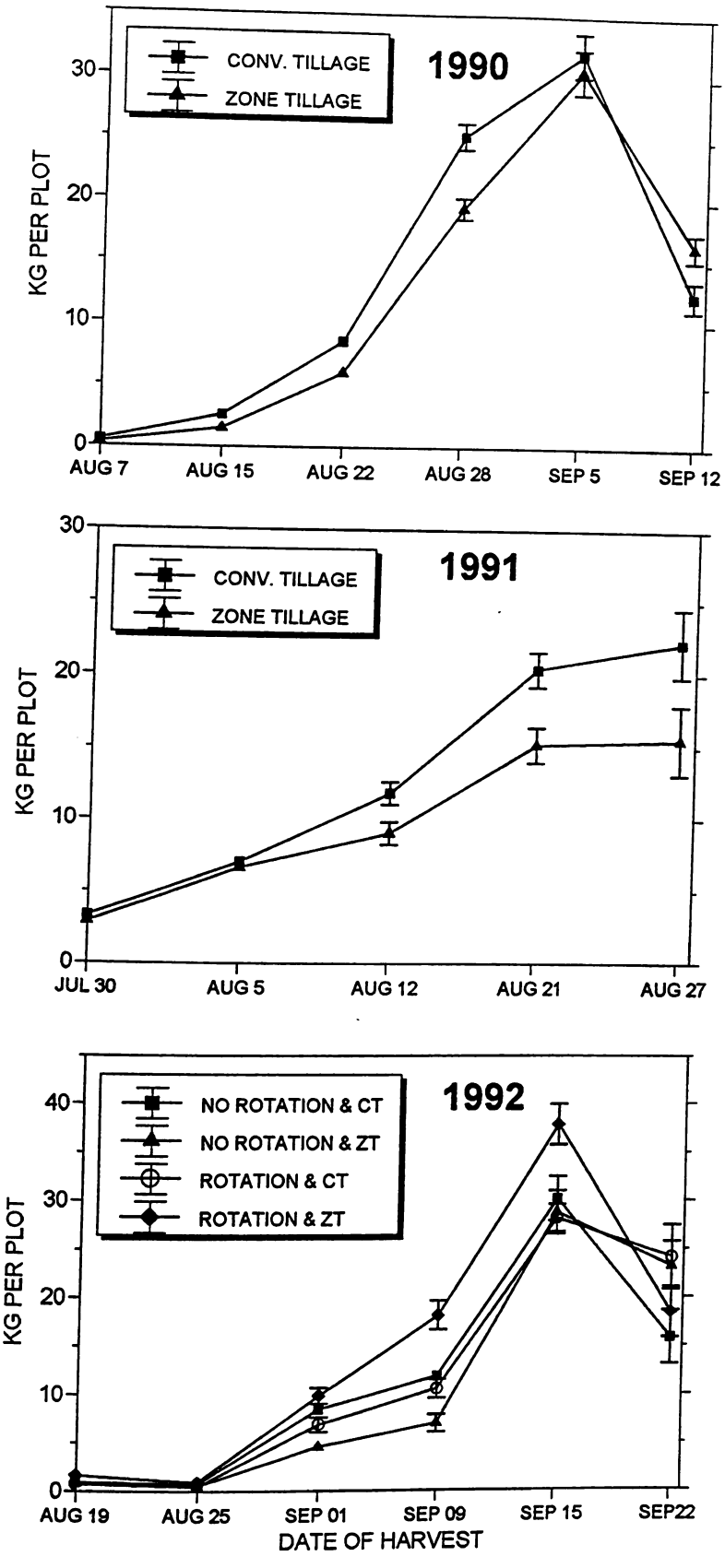
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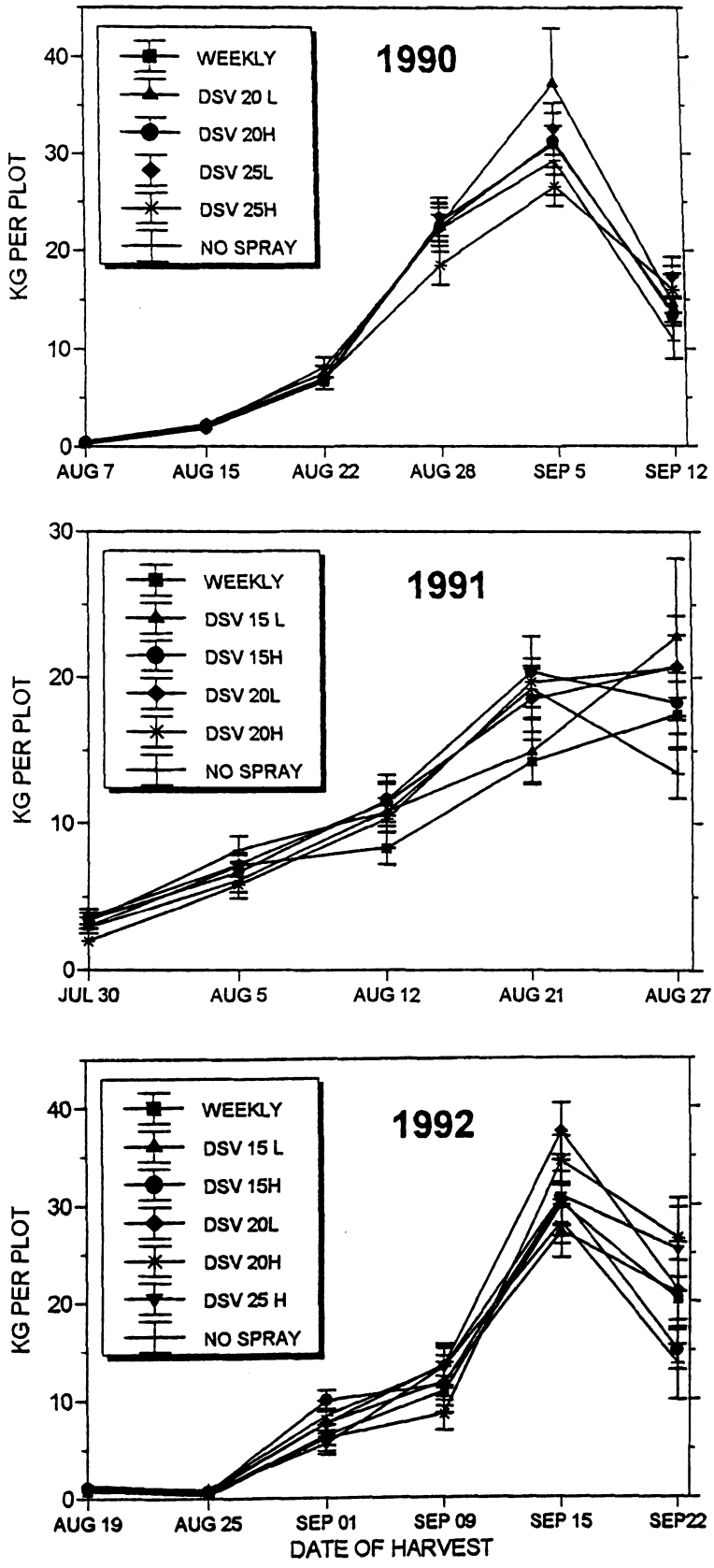
FIGURE 2: Effect of fungicide on fresh market cv. 'Pik-Rite' tomato yields harvested each week.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In the case of processing tomatoes (cv. Ohio 7870) tillage impacted the total weight of green fruit and % ripe fruit harvested (Table 5 and 6). Based on the latter observations, ZT appeared to delay fruit maturity during the 1990 season. Applications of chlorothalonil did not impact fruit yield nor fruit quality as compared to plots not sprayed (Table 5).

1991 SUMMER SEASON - Assessment of plant phenology and growth: Mean PRT height on Jun 19, 1991 was greater (P=0.05) in ZT plots (28.6 cm) as compared to plants in CT plots (26.5 cm). FMT plants were 39.8 and 36.2 cm (P=0.017) in ZT and CT plots, respectively. Number of FMT flowers was not affected by tillage. Too few flowers were set on PRT plants by Jun 19.

Tillage did not affect plant height or number of fruit set per plant of FMT and PRT as recorded on Jul 3 1991 (data not shown). Mean number of flower clusters per plant did not differ on FMT plants but were lower (P=0.007) on PRT plants in ZT plots (number = 11.0) as compared to CT plots (number = 13.4). Mean fruit diameter on FMT plots was 3.7 cm and 4.8 cm (P=0.02) in ZT and CT plots, respectively. The number of chlorothalonil sprays applied in 1990 appeared to impact tomato growth in 1991. Prior to any applications in 1991, mean PRT plant height Jun 19, 1991 in plots sprayed with 15 weekly applications in 1990 was 26.4 cm, significantly less than plant height of 29.7 cm in plots not sprayed during 1990. Plant height in plots sprayed 4 times after the accumulation of every 20 disease severity values (DSV 20H treatment) in 1990 was intermediate at 27.8 cm (LSD = 2.5 cm, 12 df, n=8). By Jul 3, differences were more pronounced. Mean plant height in the weekly (now with

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TABLE 5: Mean squares from analysis of variance for yield and fruit quality of processing tomato (PRT) in 1990.

Source of Variability	df	PRT FRUIT QUALITY AND YIELD (MT/HA)[x]			
		WT RIPE	WT GREEN	WT CULL	% RIPE
Rep.	7[y]	1290 NS	64 NS	82 NS	213 NS
Tillage (T)	1	608 NS	636 **	4 NS	572 *
Error a	7	1228	42	58	71
Fungicide (F)	6	94 NS	66 NS	64 NS	108 NS
T x F	6	372 NS	60 NS	62 NS	101 NS
Error b	84	247	44	29	61

*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for color (ripe or green) and quality (marketable fruit or culls).

[y] 1990 was the first year of the experiment resulting in 8 replications per treatment (i.e. different treatments were not applied to the main rotation plots).

TABLE 6: Effect of tillage on marketable yield of processing tomato cv. OHIO 7870 in 1990.

TILLAGE	MT/Ha			% RED
	RIPE	GREEN	CULLS	
CT	63.3	10.3	7.0	86
ZT	68.0	15.1	7.4	82
P VALUE	<0.30	0.006	<0.30	0.03

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3 applications in 1991), DSV 20H (no applications in 1991) and no spray treatments was 50.9, 56.2, and 56.8 cm, respectively (LSD=4.7 cm, 12 df, n=8). The interaction between tillage and fungicide treatment was not significant.

On Aug 9 1991, one PRT plant outside the flagged region was destructively sampled from each plot of the weekly, DSV 20H and no spray treatments. Fresh weight, total number of leaves, number of fruit, total and mean weight of fruit, and weight and percent of ripe fruit was not affected by tillage. Whole plant dry weight of plants harvested from ZT plots was less (78 gms) as compared to plants from CT plots (104 gms) (P=0.05). No difference due to fungicide treatment was observed.

Tomato fruit yield and quality 1991: Early yield was not impacted by ZT in FMT plots but by the third harvest weekly yields were less in ZT plots compared to CT plots (Figure 1). Yield decline in ZT plots was not significant at P = 0.05 for each grade size (Table 7). Reduced yield of No.1 fruit was marginally significant at P=0.06 (Table 8A). Plots sprayed weekly tended to have the lowest yields but no significant effects due to fungicide treatment were observed (Table 7, 8B).

In the case of processing tomato (cv. Heinz 8704) tillage did not affect yields (Table 9, 10). Fungicide did not affect total yields (data not shown) but analysis of variance revealed weight of green fruit was affected. One of the two control plots had more green fruit than any other treatment (data not shown).

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TABLE 7: Mean squares from analysis of variance for yield and fruit quality of fresh market tomato (FMT) in 1991.

Source of Variability	df	FMT FRUIT QUALITY AND YIELD (MT/HA)[x]				
		LARGE #1	%LRG #1	MED #1	# 2	CULL
Rep.	3[y]	84 NS	52 NS	65 NS	126 NS	25 NS
Tillage (T)	1	719 NS	227 NS	82 NS	558 NS	5 NS
Error a	3	79	89	19	119	21
Fung. (F)	6	63 NS	55 NS	16 NS	50 NS	13 NS
T x F	6	37 NS	30 NS	9 NS	38 NS	20 NS
Error b	36	40	39	12	46	15

*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for size and quality

[y] 1991 was the second year of the experiment with half the plots planted to tomato (4 reps) and half to cucumber.

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TABLE 8A. Effect of tillage on marketable yield of fresh market tomato in 1991.

TILLAGE	LARGE NO.1		MT/Ha		
	MT/Ha	%	MEDIUM NO.1	NO.2	CULL
CT	22.1	31.6	13.2	18.9	15.0
ZT	15.0	27.6	10.8	12.6	14.4
P VALUE	0.06	0.21	0.13	0.11	<0.30

TABLE 8B. Effect of fungicide treatment on marketable yield of fresh market tomato in 1991.

FUNGICIDE TREATMENT	LARGE NO.1		MT/Ha		
	MT/Ha	%	MEDIUM NO.1	NO.2	CULL
WEEKLY	16.1	29	10.3	18.4	13.2
DSV 20L	21.3	31	12.5	14.2	14.4
DSV 20H	19.8	29	12.0	18.7	15.3
DSV 25L	21.3	33	11.1	17.6	14.7
DSV 25H	20.5	32	15.0	15.0	13.0
NO SPRAY	16.6	28	11.8	13.4	16.1
P VALUE	0.19	0.23	0.19	<0.30	0.26

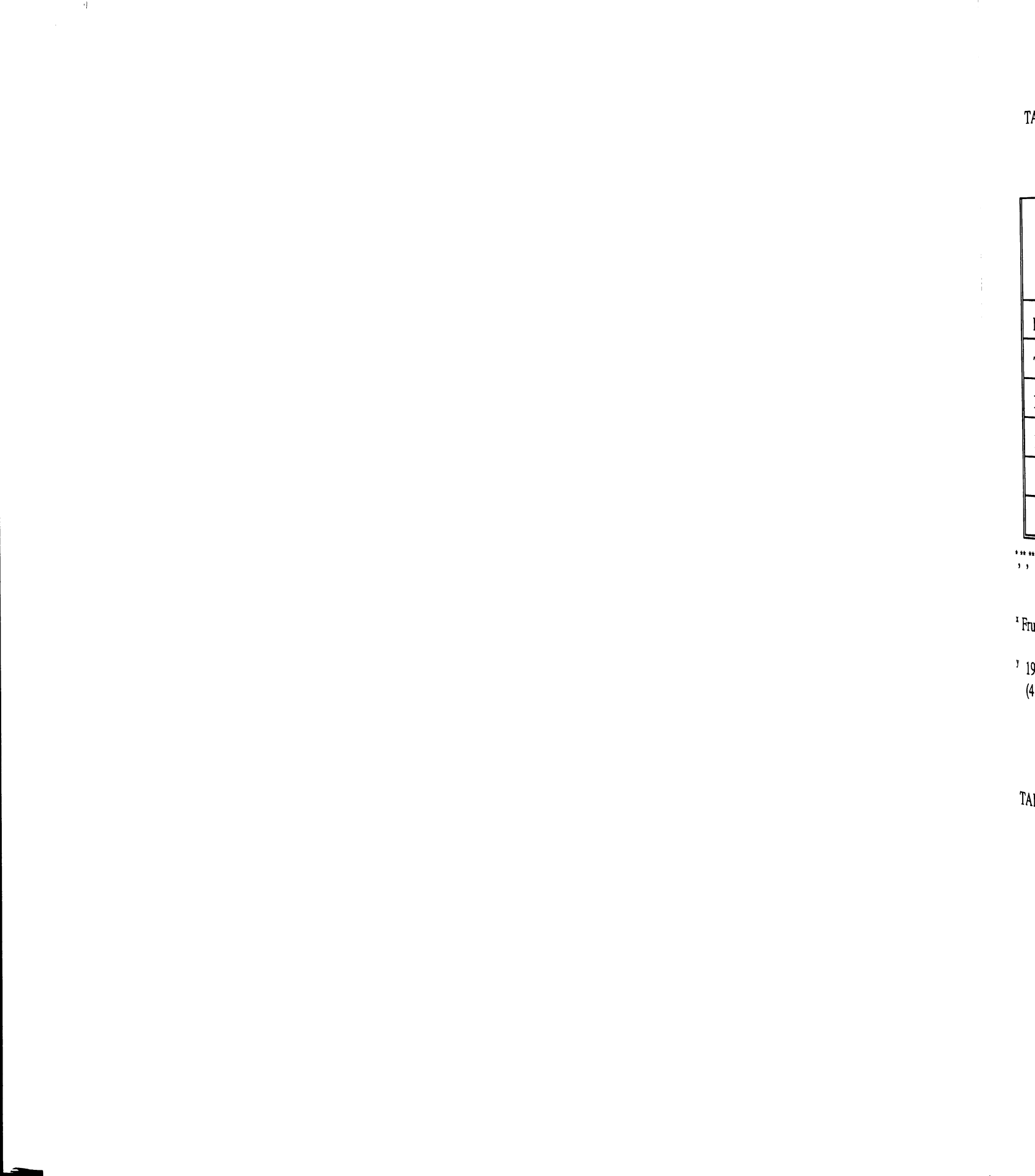

TABLE 9: Mean squares from analysis of variance for yield and fruit quality of processing tomato (PRT) in 1991.

Source of Variability	df	PRT FRUIT QUALITY AND YIELD (MT/HA)[x]			
		WT RIPE	WT GREEN	WT CULL	% RIPE
Rep.	3[y]	273 NS	251 NS	84 NS	423 NS
Tillage (T)	1	1154 NS	323 NS	42 NS	1 NS
Error a	3	861	172	23	209
Fungicide (F)	6	290 NS	122 **	19 NS	44 NS
T x F	6	61 NS	52 NS	23 *	71 NS
Error b	36	141	29	8	68

*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for color (ripe or green) and quality (marketable fruit or culls).

[y] 1991 was the second year of the experiment with half the plots planted to tomato (4 replications) and half to cucumber.

TABLE 10: Effect of tillage on marketable yield of processing tomato cv. Heinz 8780 in 1991.

TILLAGE	MT/Ha			% RED
	RIPE	GREEN	CULLS	
CT	46.9	21.1	6.5	63
ZT	37.8	16.3	4.8	63
P VALUE	<0.30	0.26	0.27	<0.30

Cucumber fruit yield 1991: The goal of the ZT system was to seed cucumber on the exact same row center where tomato once stood without further tillage inputs. However, soil pent-rometer readings in the early spring indicated a repeated ZT would likely benefit the cucumber crop. Therefore, zones were again tilled. Fungicide treatments to tomato during 1990 did not impact yield of cucumber during 1991. There-fore, data from sub-sub-plots were pooled and analyzed as a randomized complete block design with 1 factor (tillage). Cucumber yield in ZT and CT plots were similar (Table 11). Four cucumber plots were affected by a broken tile and estimated values for each grade size was determined using the MSTAT-C MISVALEST and used for analysis of variance and reporting of means.

TABLE 11: Effect of tillage on yield of pickling cucumber cv. Flurry in 1991.

TILLAGE	GRADE SIZE (MT/Ha)[v]					
	NO.1[w]	NO.2[x]	NO.3[y]	NO.4[z]	CULLS	TOTAL
CT	0.30	1.0	3.8	3.5	0.8	9.4
ZT	0.33	1.1	4.5	3.3	0.9	10.1
P VALUE	NS	NS	NS	NS	NS	NS

[v] means over CT plots include 4 estimated values for plots that were over a broken field tile. Based on the original data, mean weight of No. 3 fruit in ZT plots was significantly greater as compared to CT plots. Substituting estimated values did not affect significant differences among any other set of means including total weight.

[w] Pickling cucumbers <2.7 cm in diameter
[x] Pickling cucumbers between 2.7 and 3.8 cm in diameter
[y] Pickling cucumbers between 3.8 and 5.1 cm in diameter
[z] Pickling cucumbers >5.1 cm in diameter.

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1992 SUMMER SEASON - Assessment of plant phenology and growth: FMT mean plant height was greater (49.7 cm vs 46.4 cm; P=0.02) on Jun 18 in plots rotated with cucumber as compared to plots not rotated. No differences in other estimates of plant productivity were observed for FMT plants nor PRT plants on Jun 18. On Jul 17, mean plant height and number of flower clusters was highest in ZT x rotation treatments of PRT plants. One PRT plant was destructively sampled on Aug 13 from each plot of 3 sub-sub-treatments similar to the method described for 1991. No significant difference were observed in number or weight of fruit set, in total fresh weight or dry weight.

Tomato fruit yield and quality 1992: Rotation combined with zone tillage increased yield on specific harvest dates of FMT tomato (Figure 1). With only 2 replications (i.e. 1 df for the numerator and denominator) significant differences were not apparent for each fruit quality category (Table 12). The yields as affected by rotation and tillage are highlighted in Table 13A.

Application of fungicide impacted FMT yield on specific harvest dates (Figure 2) but no differences were observed for total yield harvested (data not shown) nor for marketable fruit. The weekly, DSV 25H and no spray treatment means are listed in Table 13B. The interaction of fungicide x rotation treatment was significant and selected results are outlined in Table 13C. The ANOVA and yield data for the full model with 4 replications is provided in Appendix A.

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TABLE 12: Mean squares from analysis of variance for yield and fruit quality of fresh market tomato (FMT) in 1992.

Source of Variability	df	FMT FRUIT QUALITY AND YIELD (MT/HA)[x]				
		LARGE #1	%LRG #1	MED #1	#2	CULL
Rep.	1[y]	69 NS	94 NS	3.3 NS	1645 NS	0.1 NS
Rotation (R)	1	682 NS	232 NS	3.7 NS	213 NS	52 NS
Error a	1	130	203	0.3	452	18
Tillage (T)	1	10 NS	28 NS	6.7 NS	288 NS	10 NS
R x T	1	17 NS	102 NS	9.8 NS	206 NS	281 *
Error b	2	73	126	3.5	199	8
Fungicide (F)	6	52 NS	33 NS	4.6 NS	113 NS	20 *
R x F	6	25 NS	32 NS	7.2 NS	47 NS	30 *
T x F	6	60 NS	40 NS	5.2 NS	29 NS	5 NS
R x T x F	6	57 NS	23 NS	4.8 NS	54 NS	12 NS
Error c	24	38	37	2.3	66	8

*, **, *** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for large #1, % large #1, medium #1, #2 and culls.

[y] nearly all sub-sub-plot treatments in 2 replications of a sub-plot treatment (conventional tillage) of a main-plot treatment (tomato rotated to cucumber) were adversly affected by a field tile that malfunctioned, resulting in poor drainage. Therefore the data were analyzed over 2 replications instead of 4 (n=56, not 112).

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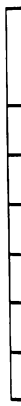

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TABLE 13A: Effect of rotation and tillage on marketable fruit of fresh market tomato in 1992.

	LARGE NO.1		MT/Ha		
	MT/Ha	%	MED #1	NO.2	CULL
NO ROTATION	18.6	26	7.9	27.2	17.0
WITH ROTATION	25.6	31	8.4	31.1	18.9
P VALUE	0.26	<0.30	0.16	<0.30	<0.30
CT	21.7	29	7.8	26.9	17.5
ZT	22.5	28	8.5	31.4	18.4
P VALUE	<0.30	<0.30	0.30	<0.30	<0.30

TABLE 13B. Effect of fungicide treatment on marketable yield of fresh market tomato in 1992.

FUNGICIDE	LARGE NO.1		MT/Ha		
	MT/Ha	%	MED#1	NO.2	CULL
WEEKLY	22.6	30.9	6.9	29.0	14.9
DSV 25H	25.2	30.8	8.0	30.5	18.4
NO SPRAY	19.3	26.5	8.3	23.5	19.2
P VALUE	0.26	<0.30	0.10	0.16	[y]

[y] Rotation x Fungicide interaction was significant.

TABLE 13C: Means of cull fruit of selected fungicide x rotation treatment interactions.

	CULLS MT/Ha	
	NO ROTATION	WITH ROTATION -
WEEKLY	16.8	13.0
DSV 25H	13.9	22.9
NO SPRAY	18.9	19.5
LSD (n=4)	4.0	

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Ethrel was applied on PRT plants Sep 14 to enhance ripening. However, cool weather persisted and Ethrel appeared to have little impact. The experiment was prematurely terminated on Sep 29 to avoid forecasted frost and loss of yield data. The grand mean of percent ripe fruit was 24.5%. Therefore, total yield is presented instead of marketable ripe fruit. Total yield in rotation plots was 43.3 MT Ha^{-1} as compared to 47.2 MT Ha^{-1} in plots planted to continuous tomato (P=0.27). ZT increased yield in combination with all other treatments. The mean yield was 53.0 MT Ha^{-1} in ZT plots and 37.4 MT Ha^{-1} in CT plots (P=0.04). All interactions were not significant. Yield was less in plots not sprayed (35.1 MT Ha^{-1}) as compared to plots sprayed weekly with chlorothalonil (mean = 48.9 MT Ha^{-1}) and the DSV 25H treatment (48.2 MT Ha^{-1}), but the F-ratio for fungicide effect was marginally significant at P=0.06.

DISCUSSION

The zone tillage system enhanced or did not affect total yield of fresh market and processing tomato in 1990, did not affect yield of cucumber planted on the exact same row center in 1991, and enhanced yield of tomato in 1992 when combined with rotation. Our goal was to perform no additional tillage operations from the spring of 1990 to the summer of 1991. However, soil density readings indicated benefit would likely occur with a repeat tillage of the zone prior to planting cucumber. Never-the-less, compared to a conventional tillage system, 4 CT operations were eliminated (Figure 1 of Chapter 2). Immediately after the cucumber harvest, a window of opportunity for a summer fallow (Sarrantonio 1992) was exploited to seed a green mustard crop. The crop was chosen for its possible ability to reduce inoculum of plant

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pathogens (Mojtahedi et al. 1993; Muehlchen et al. 1990), for its rapid growth to protect the light sandy soils from erosion, and for its potential ability to tie up nutrients that other wise may leach from the soil (Sarrantonio 1992). The crop was included as part of a production system and the experiment was not designed to determine potential benefits of the mustard crop. After the mustard crop, complete conventional tillage was performed. In this manner, as opposed to continuous no-till, weed, insect and pathogen life cycles can be disrupted on a biennial cycle to reduce the potential of serious build up of pests in the vegetable production system. Complete no-till systems may be unrealistic for vegetable crops.

The zone tillage system also provided a surface rye residue that persisted for the entire cropping season. Most advantages associated with no-till would therefore apply to this zone tillage system including reduced water and wind soil erosion, reduced wind-wiping of plants, and enhanced water use efficiency, etc (Coolman and Hoyt 1993; Gebhardt et al. 1985; Phillips et al. 1980). Likewise, the known benefits of rye were exploited to smother weed and, through allelopathic substances, delay early season weed emergence and the need for early season herbicide (Putnam 1990; Wallace and Bellinder 1992). Most important, the preparation of strips early in the season, combined with zone tillage, circumvented problems commonly associated with no-till systems in vegetable production (Doss et al. 1981; Knavel et al. 1977; Price and Baughan 1988). For example, Price and Baughan (1988) have shown tomato transplants set into no-till plots are less productive than plants in conventional tillage (CT) plots. Reduced productivity may be due to allelopathic substances released by rye residue, high carbon to nitrogen ratio limiting early season nitrogen availability, poor root to soil contact of the plant, or possibly high populations of parasitic pathogens

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that persist in association with rye residue. This ZT system ensures rye residue does not accumulate in strips where plants are to go. Early season kill of strips also allows time for decomposition of the residue that overwinters and potential disadvantages are avoided.

ZT fractures the soil where plants are to go and ensures compaction is not a problem. ZT loosens the soil for enhanced root exploration and subsequent plant productivity (Grajauskis 1990). In 1990, ZT allowed for total yields of both fresh market and processing tomato similar to a CT system. In 1991, initial measurements on Jun 19 indicated enhanced plant productivity in ZT plots. However, by Jul 3, parameters of plant productivity began to indicate ZT plants lagged behind CT plants. On Aug 9, total dry weight of plants harvested from ZT plots was significantly less as compared to CT effects. Ultimately, yields were substantially reduced (Figure 1). The positive effect of ZT in 1991 on early plant productivity is supported by similar initial yields in ZT and CT plots for the first two FMT harvest dates, after which time weekly yields in CT plots surpassed yields in ZT plots (Figure 1). The decline in plant productivity can be associated with the lack of rotation. Plants in ZT plots had a high incidence of collar rot (Chapter 2). Lesions completely girdled stems, a problem known to decrease plant productivity (Jones et al. 1991). Also, lack of tillage allowed for persistence of weeds which became problematic during the growing season.

Applications of chlorothalonil on a reduced schedule, according to the TOMCAST model, can reduce control of defoliation due to early blight and, especially at reduced rates of fungicide, can increase the incidence of fruit mold (Chapter 2) as compared to plots sprayed weekly. However, reduced sprays rarely affected marketable yield of processing and fresh market tomato. One notable exception occurred with

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FMT in 1990. CT combined with weekly sprays limited weight of culls most substantially compared to other fungicide treatments. The high incidence of culls had been associated with a high incidence of soil rot, caused by *Rhizoctonia solani* (Chapter 2). Soil rot problems did not persist and were controlled with a reduced-sprays program in subsequent years (Chapter 2).

Lack of association of yield decline with defoliation and amount of fungicide sprays has been noted by others (Brammall 1993; Ferrandino and Elmer 1992). Brammall demonstrated marketable yield was not impacted by chlorothalonil, applied according to the TOMCAST model, as compared to plots not sprayed. However, in our study, plots not sprayed tended to have the lowest marketable yield and on occasion, such as PRT yields in 1992, were close to significance. Recommendations not to spray would introduce considerable risk at this time. However, if lines with superior genetic resistance can be incorporated into the production system in the future, cultural and genetic control may suffice.

Fungicide applications on a weekly schedule never resulted in highest total yield (Figure 2). In fact, plants in plots sprayed weekly were stunted in 1991 and yield tended to decrease. Fifteen fungicide sprays were applied the preceding year to the same plots. However, evidence proving a direct link was not acquired.

The ZT system outlined here has considerable potential for vegetable production systems. Additional research is required to determine optimum rotation cycles, the potential of using ZT with other crops, and to build up a larger knowledge base that will enable growers to flexibly manage on farm inputs for enhanced crop productivity and profitability.

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CHAPTER IV

MAJOR DISTINCTIONS IN GENOMIC STRUCTURE DETECTED BY REP-PCR FINGERPRINTING SEPARATE STRAINS CLASSIFIED AS *XANTHOMONAS CAMPESTRIS* PV. VESICATORIA INTO AT LEAST FOUR GROUPS.

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ABSTRACT

DNA primers corresponding to repetitive sequences (REP, BOX and ERIC elements) and the PCR (rep-PCR) were used to generate complex fingerprint patterns that identified 4 distinct groups among strains classified as *Xanthomonas campestris* pv. vesicatoria. These groups were differentiated by near complete dissimilarity in migration rates of 60 or more bands generated with rep-PCR. Group A isolates originated from tomato or pepper. Most of these isolates proved to be negative in starch hydrolysis and pectolytic activity tests. All Group A isolates were found to be relatively homogenous with regard to their rep-PCR fingerprinting patterns. Group B isolates originated primarily from tomato and were positive for starch hydrolysis and pectolytic activity. Group B strains were found to comprise an important component of the tomato spot complex in the northcentral tomato production region of North America. One isolate was classified as a Group C strain. Two isolates were classified as Group D strains and one such isolate was found to be highly virulent to tomato. Interestingly, group D strains were found to share numerous bands of similar mobility with strains pathogenic for cabbage, classified as *Xanthomonas campestris* pv. campestris, suggesting the group D strains are closely related to the cabbage pathogen.

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INTRODUCTION

Xanthomonas campestris pv. vesicatoria (Xcv), the causal agent of bacterial spot on pepper and tomato, was first diagnosed in the early 1920's (Doidge 1921; Gardner and Kendrick 1921; Higgins 1922) and occurs worldwide in regions of pepper and tomato production (Hayward and Waterson 1964; Sherf and MacNab 1986). On tomato, Xcv affects all above ground plant tissue and can incite marketable yield losses from 5 to 70% (Pohronezny and Volin 1983; Sherf and MacNab 1986). Chemically based and cultural practices are currently the primary farm-level disease management strategies. However, routine application of bactericides, such as copper or streptomycin, do not provide consistent control, because of low efficacy (Hausbeck and Kusnier III 1993) and the ability of populations to acquire resistance to the bactericides (Minsavage et al. 1990; Stall et al. 1986). Cultural practices such as burial of crop debris, crop rotation and use of windbreaks to limit on-farm incidence of spot in tomato has been recommended (Jones et al. 1991; Sherf and MacNab 1986) and implemented, but nevertheless throughout the northcentral region of North America (MI, OH, IN, U.S.A. and Ontario, Canada), spot problems recur each year. Farm level integrated disease management practices appear to have minimal impact on disease control, especially when weather conditions favor the spread of disease. Ultimately, disease control is likely to be achieved primarily through disease management strategies implemented before the seed (or transplants) arrive at the farm, such as the development of genetic resistance and implementation of protocols designed to limit the introduction of the initial inoculum (Goode and Sasser 1980).

However, breeding for durable disease resistance and implementing necessary detection/diagnostic protocols have posed a challenge because Xcv is phenotypically,

serologically, pathogenically and genotypically diverse (Doolittle and Crossan 1959; Dye 1962; Dye et al. 1964; Jones et al. 1993a; Minsavage et al 1990.; Klement 1959; Stall et al. 1993; Sutic 1959; Vauterin et al. 1990; 1991; Wang et al. 1990; Whalen et al. 1988). For example, genetic resistance was developed in tomato (Scott et al. 1989), but a strain from Argentina and virulent for the resistant host was already identified (Wang et al. 1990) before the resistance was commercially deployed.

Techniques that emphasize overall chromosomal organization of Xcv may help elucidate our understanding of the genotypic structure of natural populations and may provide a framework for understanding the evolutionary dynamics of pathogenesis and optimal mathods for the implementation of integrated disease management strategies, including diagnostic protocols, plant breeding programs, and the deployment of genetic resources. Therefore, this research was initiated using a genomic DNA fingerprinting approach to determine the genetic diversity of Xcv isolates obtained from diverse geographic regions. This rapid and highly reproducible method employs primers corresponding to repetitive extragenic sequences [repetitive extragenic palindromic (REP) sequences (Gilson et al. 1984; Higgins et al. 1982), enterobacterial repetitive intergenic consensus (ERIC) sequences (Hulton et al. 1991; Sharples and Lloyd 1990), and the BOX element (BOX1A sequences) (Martin et al. 1992)] to generate complex fingerprint patterns from DNA of bacteria in combination with the polymerase chain reaction (PCR) protocol (Versalovic et al. 1991; de Bruijn 1992; Koeuth et al. 1993). The technique, known as REP-PCR, ERIC-PCR and BOX-PCR, respectively (and rep-PCR collectively), distinquishes *Xanthomonas* and *Pseudomonas* strains at the pathovar and subpathovar level (see Appendix D), presumably based on overall chromosomal organization.

This chapter highlights the detection of 4 distinct groups among isolates classified as *Xanthomonas campestris* pv vesicatoria. The groups correlate with selected phenotypic characteristics such as amylolytic and pectolytic activity, highlighting the utility of rep-PCR in distinguishing phytopathogenic bacteria at the pathovar and sub-pathovar level. This chapter also shows that amylolytic/pectolytic strains [subgroup "B" strains sensu Vauterin et al. (1990) and Group "B" or T2 strains sensu Jones et al. (1993)] constitute an important component of the tomato spot complex in the northcentral production region of North America.

MATERIALS AND METHODS

BACTERIAL ISOLATES AND CULTURE CONDITIONS. MSU accession numbers, original strain designation(s), geographic origin, year of isolation, race designations and sources of bacterial isolates or genomic DNAs are listed in Table 1. Xcv suspensions initiated from single colonies were stored at -70 C in 15% glycerol and re-streaked on nutrient-yeast-dextrose agar (NYDA) (Jones et al. 1981) as required. Bacterial cells were grown for DNA isolation from single colonies in 40 ml LB for 24 to 48 hr at 27°C on a rotary shaker (200 rpm).

ISOLATION OF CHROMOSOMAL DNA AND PCR CONDITIONS. Total genomic DNA was prepared as described in Appendix D. The DNA sequence of the primers employed and the PCR conditions used were also described in Appendix D. PCR amplification was performed in a model 110s Tempcycler II (Coy Corporation, Grass Lake, MI) or a Perkin & Elmer thermocycler, using the following cycles: 1

Table 1: Bacterial isolates or DNA used in this study and associated information.

MSU ID #	STRAIN ID	HOST	LOC-ATION	YEAR	GROUP	RACE	STARCH	PECT. ACTIV. CVP	CKTM	Source or Ref.
666	Xv 36		FL		A		-	-	T	JBJ
684	Xv 29	P	OK	1990	A		-	-	P	CLB
685	Xv 31	P	OK	1989	A		-	-	P	CLB
686	Xv 334	P	CAR		A	1	+	-	V	JBJ
687	Xv 85	T	FL		A		±	-	T	JBJ
688	Xv 1	P	FL		A		±	-	T	JBJ
689	Xv 858	T	MX		A	2	-	-	T	JBJ
690	Xv 104	P	TW		A	3	±	-	P	JBJ
691	Xv91	P	TW		A	3	-	-	V	JBJ
692	Xv 89	P	TW		A	3	-	-	P	JBJ
694	91W13	P	ONT	1991	A		-	-	P	RB
697	Xv 110	P	TW		A	1	-	-	P	JBJ
698	Xv 855	T	MX		A	2	-	-	T	JBJ
699	Xv 856	T	MX		A	2	-	-	V	JBJ
700	Xv 857	T	MX		A	2	-	-	T	JBJ
701	Xv 859	T	MX		A	2	±	-	V	JBJ
702	Xv 18	T	FL		A		±	-	V	JBJ
703	Xv 122	T	TW		A		-	-	T	JBJ
704	Xv 300	T	CAR		A		-	-	T	JBJ
706	Xv 531	T	CAR		A	2	-	-	T	JBJ
739	Xv 597	P	CAR		A		-	-	P	JBJ
740	Xv 102	P	TW		A	1	-	-	P	JBJ
741	Xv 63	P	FL		A		±	-	P	RES
742	Xv 92-17	P	FL	1992	A	2	-	-	P	RES
744	Xv 90-1P	P	GA	1990	A		-	-	P	RG
745	Xv 89-53P	P	GA	1989	A		-	-	P	RG
746	Xv 89-52P	P	GA	1989	A		±	-	P	RG
747	Xv 88-45P	P	GA	1988	A		-	-	P	RG
748	Xv NC		GA		A		-	-	T	RG
837	SS-Pepper	P-GH	ONT	1992	A		-	-	P	DHAN
865	TS 8	T	ONT	1990	A		-	±	P	DHAN
866	TS 16	T	ONT	1990	A		-	±	P	DHAN
867	TS 26	T	ONT	1990	A		-	±	P	DHAN
868	TS 31	T	ONT	1990	A		-	-	P	DHAN
869	TS 35	T	ONT	1990	A		±	-	P	DHAN
878	Sp2-92	P-S	GA	1993	A		-	-	P	GO
879	Sp66-92	P-S	GA	1993	A		-	-	P	GO
880	Sp124-92	P-S	GA	1993	A		±	-	P	GO
882	Sp133-92	P-S	GA	1993	A		-	-	P	GO
883	Sp135-92	P-S	GA	1993	A		-	-	P	GO
884	P93-DIA	P-GH	GA	1993	A		±	-	P	GO
886	Xv 18 (OH)	P	OH	1992	A	1	-	-	P	SM
887	Xv 47	P	OH	1992	A	1	-	-	P	SM
888	Xv 44	P	OH	1992	A	1	-	-	P	SM
890	Xv 71	P	OH	1992	A	1	-	-	P	SM
908	ATCC 11633	P	NJ	1947	A		-	-	V	ATCC
931	Xv 931	P-GH	MI	1993	A		-	-	P	THIS STUDY
939	Xv 939	P	MI	1993	A		-	-	P	THIS STUDY
943	Xv 93-1		FL	1993	A	3	-	-	V	RES
944	Xv 93-26		FL	1993	A	T1	-	-	T	RES
945	Xv 93-24		FL	1993	A	2	-	-	-	RES
947	Xv 92-16		FL	1992	A	1	-	-	P	RES
948	Xv 75-3		FL	1975	A	T1	±	-	V	RES*
949	Xv 93-29		FL	1993	A	T1	-	-	T	RES
	LMG 905			1982	A		NA	NA	NA	JS**
	LMG 910	P	MOROCCO	1976	A		NA	NA	NA	JS**
	LMG 929	P	FL	1969	A		NA	NA	NA	JS**

Table 1 Continued...

MSU ID #	STRAIN ID	HOST	LOC-ATION	YEAR	GROUP	RACE	STARCH	PECT. ACTIV. CVP	CKTM	Source or Ref.
682	Xv 10	T	OK	1987	B		+	+	subtle	CLB
683	Xv 15	T	OK	1987	B		+	+	-	CLB
736	Xcv 736	T	MI	1992	B		+	+	-	THIS STUDY
833	DC92-13	T	ONT	1992	B		+	+	-	DC
834	DC92-21	T	ONT	1992	B		+	+	-	DC
835	DC92-23	T	ONT	1992	B		+	+	-	DC
859	Xcv 859	T	MI	1991	B		+	+	-	THIS STUDY
870	CC 164#3	T	ONT	1992	B		+	+	-	DHAN
871	CC 195#1	T	ONT	1992	B		+	+	-	DHAN
872	TS 1	T	ONT	1979	B		+	+	-	DHAN
907	ATCC 35937	T	NZ	1955	B		+	+	-	ATCC**
909	ATCC 11551	T	IN	1943	B		+	+	-	ATCC
918	ICBB 167	T			B	T2	+	+	subtle	JBJ
919	BA 27-1	P	ARG		B	T2P3	+	+	-	JBJ***
920	BA 29-1	T	ARG		B	T2P3	+	+	-	JBJ***
921	BV 5-3A	T	ARG		B	T2	+	+	-	JBJ***
923	BV 6.1	T	ARG		B	T2	+	+	-	JBJ
924	BV 7.3A	T	ARG		B	T2	+	+	subtle	JBJ
925	BV 4.1	T	ARG		B	T2	+	+	-	JBJ
969	Xv 56	T	BRAZIL		B	T2	+	+	-	JBJ***
	LMG 920	T	ITALY		B		NA	NA		JS**
981	Xcv 981	T	MI	1993	B					THIS STUDY
982	Xcv 982	T	MI	1993	B					THIS STUDY
705	Xv 441	T	CAR		C	1	-	-	subtle	JBJ
832	DC 92-6	T-GH	ONT	1992	D		+	+	T	DC
857	DC 91-1	T-GH	ONT	1991	D		+	+	T	DC
803	X-1	geran	KS	1986			-	-	T	KD
910	5-2-4	geran	ISRAEL	1987			-	+	-	MD
942	Xpel 942	geran	MI	1993			-	+	-	THIS STUDY
805	Xp 805	bean	MI	1992			+	-	V	LA
806	JT1	cabbage	MI	1990			+	+	-	JT
807	JT4	arabid	MI				+	+	T	JT
898	Xcc 898	cabbage	MI	1991			+	+	-	THIS STUDY
876	Pss 11	Cherry	MI				-	-	-	AJ
894	Pss 11	Cherry	MI				-	-	-	AJ
895	Pss 66	Cherry	MI				-	-	-	AJ
896	Pss 19	Cherry	MI				-	-	-	AJ
915	Pst 915	T-GH	MI	1993			-	-	-	THIS STUDY

TABLE 1 CONTINUED...

Footnotes to Table 1:

Host: P = Pepper S = Seed geran = Geranium
T = Tomato GH = Greenhouse aradib = arabidopsis

Location: FL = Florida; GA = Georgia; OK =Oklahoma;
MI = Michigan; ONT = Ontario; NJ = New Jersey;
OH = Ohio; KS = Kansas; NZ = New Zealand;
Moro = Morocco; ARG = Argentina; TW = Taiwan;
CAR = Caribbean; MX = Mexico.

Race: (after Minsavage et al. 1990)
T1 = tomato race 1
1 = pepper race 1
2 = pepper race 2 (P2)
3 = pepper race 3 (P3)
T2 = tomato race 2 (after Wang etal. 1990)

Starch: (-) = not able to hydrolyze starch
(±) = hydrolyzed starch weakly
(+) = hydrolyzed starch extensively

Pectolytic activity on CVP medium: (-) = no activity
(+) = pectolytic
(±) = slight activity

CKTM: P = formed a clear ring, "pepper type"
T = formed opaque white precipitate "tomato type"
V = intermediate phenotype; - = no phenotype on CKTM

Source or reference: JBJ=J.Jones, Gulf Coast Research Center, University of Florida; CLB=C. Bender, Department of Plant Pathology, Oklahoma State University; RB=R.Brammal, Ontario Ministry of Agriculture and Food, Simcoe, Ontario; RES=R. Stall, Department of Plant Pathology, University of Florida; RG=R.Gitaitis, Department of Plant Pathology, University of Georgia; DHAN=Dr. Dhanvantari, Agriculture Canada, Harrow, Ontario; GO=G.O'Keefe, Georgia Dept. Agriculture, Georgia; SM=S.Miller, Florida Dept. Agr. and Consumer Serv., Gainesville; ATCC=American Type Culture Collection; JS=J.Swings, Laboratorium voor Microbiologie, Universiteit, Gent, Belgium; DC=D.Cuppels, Agriculture Canada, London, Ontario; KS=K.Dunbar, Department of Botany and Plant Pathology, Michigan State University; MD=M.Daughtrey, Long Island Hort. Res. Lab.; LA=L.Afanador, Department Crop and Soil Sciences, Michigan State University; AJ=A.Jones, Department of Botany and Plant Pathology, Michigan State University; JT=J.Tsuji, DOE-Plant Research Laboratory, Michigan State University; references * Whalon et al. 1988; ** Vauterin et al. 1990,1991; *** Beaulieu et al. 1991 and/or Stall et al. 1994.

initial cycle at 95°C for 7 min; 30 cycles at 94°C for 1 min, annealing at 44°C, 52°C or 53°C for 1 min with REP, ERIC and BOX primers, respectively, and extension at 65°C for 8 min with a single final extension cycle at 65°C for 15 min, followed by a soak at 4°C. PCR mixtures were overlain with 25 ul of mineral oil (Sigma M3516). Each PCR experiment included a control (no template DNA) and one or more controls with DNA from another pathovar of *X. campestris*.

Approximately 6 ul of PCR generated DNA fragments were resolved by gel electrophoreses at 4°C in 1.5% agarose gels in 0.75X or 0.5X TAE buffer at 5 V/cm. Differences in fingerprint patterns between groups were assessed visually.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION. All isolates were evaluated by selected phenotypic tests, commonly used to characterize Xcv strains (Gitaitis et al. 1987). Starch utilization on nutrient agar (Difco Laboratories, Detroit, MI), amended with 1% soluble starch, was scored qualitatively as weak (±), positive (+) or negative (-). After 2 to 3 days of bacterial growth, starch utilization was considered negative if no clear zone formed when the medium was stained with an iodine solution. Quantitative estimates of starch utilization were obtained for isolate Xcv 736, ATCC 35937, Xv 75-3, and ATCC 11633 by dipping a 13 mm sterilized filter disk in a bacterial suspension (10^8 cfu/ml) and placing the disk on 15 ml of the starch medium in the center of 9 cm petri dishes. Cleared zones in three samples of each isolate were measured at 18 to 178 hrs after inoculation of the medium. The linear portion of the data was expressed as starch hydrolysis in mm per hour based on linear regression analysis.

Pectolytic activity was determined by growth on crystal violate pectate medium

(CVP) (Cuppels and Kelman 1974) and cellulolytic activity was determined on carboxymethylcellulose medium (CMC) (Gitaitis et al. 1991). Colony characteristics on a basal CKTM medium lacking antibiotics were recorded as described by Sijam et al. (1992). Xcv isolates form a precipitate on CKTM and can therefore easily be identified on this selective medium. Isolates from tomato develop opaque white halos whereas isolates from pepper simply form a clear ring (Sijam et al. 1992).

Bacterial cultures were initiated on NYDA and cells were transferred to each medium with an inoculating loop. All physiological and biochemical tests were repeated a minimum of two times for each isolate. The quantitative experiment for starch utilization was conducted once.

RESULTS

FOUR GENOTYPES ARE RESOLVED BY REP-, BOX- AND ERIC-PCR.

DNA fingerprints were generated from total chromosomal DNA extracted from over 80 isolates of Xcv originating from various parts of the world (Table 1). Primers corresponding to REP, BOX and ERIC sequences, in combination with the polymerase chain reaction, generated complex genomic fingerprinting patterns from DNA of each isolate of up to 20 or more PCR products, that ranged in size from approximately 0.2 to over 5 kb. Isolates were classified into 4 distinct groups based on these fingerprinting patterns (Figure 1). The REP- (lanes 1 to 6), BOX- (lanes 7 to 12), and ERIC-PCR (lanes 13 to 18) experiments were equally effective in delineating the four groups. Group A (lanes 1,7,13), Group B (lanes 2,8,14), Group C (lanes 3,9,15) and Group D (lanes 4&5, 10&11, 16&17) included 56 (69%), 25 (31%), 1 (1.2%) and 2

(2.4%) of the isolates evaluated, respectively. Not one rep-PCR generated band common to all groups was generated by the REP-, BOX-, or ERIC-PCR experiments. With the ERIC-PCR experiment, one to 3 bands appeared to comigrate among isolates classified as Group A or Group B. For example, isolate ATCC 11633 (Figure 1, lane 13) appeared to have 3 bands (highlighted by arrowheads in lane 13) that comigrated with bands generated from chromosomal DNA of Xcv 736 (Figure 1, lane 14). Sequencing or hybridization studies would need to be conducted to determine if the comigrating bands are analogous portions of DNA in both the Group A and B isolates.

The rep-PCR experiments effectively differentiated 4 groups among strains classified as *Xanthomonas campestris* pv. vesicatoria based on total chromosomal fingerprint patterns. Disparate fingerprint profiles between the four groups, categorized here as Group A,B, C and D, suggest that the groups are genetically highly dissimilar. Fingerprints generated from Xcv strains were unique as compared to fingerprint profiles generated from over 30 other xanthomonads (Appendix D and data not shown), and numerous strains classified as *Pseudomonas*, *Clavibacter*, as well as saprophytic bacteria associated with field tomato plants, greenhouse tomato plants and overwintered tomato debris (data not shown).

GENOTYPIC VARIATION WITHIN GROUP A AND B AS DETERMINED BY BOX, REP-, AND ERIC-PCR.

In contrast to the near complete different fingerprint patterns between groups, rep-PCR fingerprint profiles generated from DNA of isolates within each group were highly similar (Figure 2 and 3).

FIGURE 1. Agarose gel electrophoresis of fingerprint patterns obtained from genomic DNA from isolates of *Xanthomonas campestris* pv. vesicatoria using primers corresponding to REP (lanes 1 to 6), BOX (lanes 7 to 12) and ERIC (lanes 13 to 18) sequences. Six ul of PCR products were loaded in each lane. A typical Group A pattern (lanes 1,7 and 13), Group B pattern (lanes 2,8 and 14), the Group C pattern (lanes 3,9 and 15) and Group D pattern (lanes 4&5, 10&11 and 16&17) are displayed. The right- and left-most lane contain DNA size markers (1 kb ladder, Gibco-BRL) indicated in base pairs. Arrowheads identify similarities or differences among selected isolates as outlined in the text. PCR bands were resolved on 1.5% agarose gels stained with ethidium bromide.

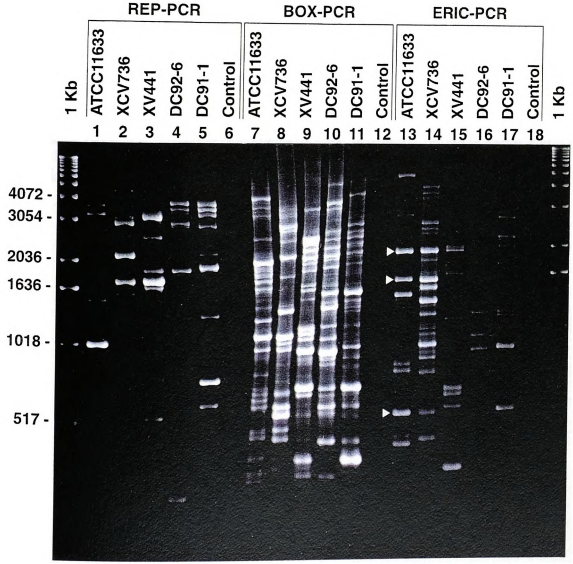

REP-PCR
BOX-PCR
ERIC-PCR
1 Kb
ATCC11633
XCV736
XV441
DC92-6
DC91-1
Control
ATCC11633
XCV736
XV441
DC92-6
DC91-1
Control
ATCC11633
XCV736
XV441
DC92-6
DC91-1
Control
1 Kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
4072 -
3054 -
2036 -
1636 -
1018 -
517 -

FIGURE 2. Agarose gel electrophoresis of fingerprint patterns obtained from genomic DNA from isolates of *Xanthomonas campestris* pv. vesicatoria Group A (Top) and Group B (Bottom), using primers corresponding to BOX sequences. Other details are as outlined in the legend of Figure 1.

1 Kb
ATCC11633
LMG905
TS35
TS8
XV93-29
XV93-26
XV44OH
91W13
XV85
XCV939
XCV931
SP135
SP133
XV89
XV334
XV104
XV531
XV18
XV1
XVNC
XV36
XV300
XV859
XV856
XV855
XV75-3
XV63
XV122
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
4072
3054
2036
1636
1018
517

1 Kb
TS1
XV10
XV16
XCV736
XCV859
DC92-13
DC92-21
DC92-23
CC164
CC195
ATCC35937
ATCC11551
ICBB167
LMG920
XCV981
XCV982
BV6-1
BV4-1
XV56
BA27-1
BA29-1
BV5-3A
1 Kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

BOX-PCR differentiated 15 BOX-PCR fingerprint types within Group A. Patterns were highly similar (Figure 2 top) with differences limited to the presence or absence of 1 to 3 bands, as compared to the predominant pattern highlighted by ATTCC 11633 (lane 1). For example, LMG905, TS35, TS8 and Xv93-29 (lanes 2 to 5) each yielded an extra single band of approximately 700 bp (opposing arrows in lanes 2 and 5). Likewise, the isolates Xcv939, Xcv931, Sp135, Sp133, Xv89, Xv334 and Xv104 (lanes 10 to 16) yielded a polymorphic band about 950 bp in size (opposing arrows in lanes 10 and 16). However, each of the latter isolates were not completely similar. For example, isolate Xv334 did not yield two bands in the 3 kb range and possessed a distinct polymorphism at 600 bp. Examples of other polymorphisms generated from other isolates are highlighted by arrowheads.

BOX-PCR delineated 5 patterns among strains classified as Group B (Figure 2 bottom, polymorphisms highlighted by arrowheads). The distinct BOX-PCR fingerprinting patterns could not be associated with geographic region. For example TS1 originated in Ontario in 1979 and had a BOX-PCR fingerprint indistinguishable from the two Oklahoma isolates (Xv10 and Xv15,1987) and two isolates from Michigan (Xcv859 from 1991 and Xcv736 from 1992; see Figure 2B, lanes 1 to 5). Likewise, Xcv 981 and 982, isolated from different Michigan fields of processing tomato in 1993, had BOX-PCR fingerprint patterns identical to BV6-1 and BV4-1 isolated in Argentina. Xv56 was also similar to the latter 4 isolates. The date of isolation also did not appear to affect the rep-PCR fingerprint profiles of Xcv strains. ATCC 35934 is the pathovar reference strain isolated from New Zealand in 1955 and could not be distinguished from 5 strains (DC92-13, DC92-21, DC92-23, CC164 and CC195) isolated from independent epidemics in Southwest Ontario in 1992 (see lanes

6 to 14). BA27-1 and BA29-1 (lanes 20 and 21) were also indistinguishable from each other. BV5-3A generated a unique profile (lane 22).

ERIC-PCR differentiated 13 fingerprint types within Group A. As with BOX-PCR, patterns were highly similar with differences limited to the presence or absence of 1 to 3 bands (Figure 3). Arrowheads highlight several of the unique characters that were scored. For example, Xv18, Xv531, Xv104 and Xv334 each lacked a prominent 1.9 Kb band (lanes 10 to 13 highlighted by opposing arrows). Xv334 (lane 13) was the least similar to the other memebers of Group A with regard to fingerprint patterns generated.

ERIC-PCR fingerprinting lead to the inclusion of the Group B strains into the exact same clusters as BOX-PCR. The 5 unique banding patterns displayed abundant common bands (Figure 4A, lanes 1 to 5) but numerous additional bands generated strain specific fingerprint profiles.

The relatively low total number of bands generated by REP-PCR rendered the REP primer set the least useful for discriminating among strains within Group A. All isolates within Group A generated REP-PCR fingerprints identical to ATCC11633 (see Figure 1, lane 1) with two exceptions (Figure 5). Xv334 yielded two additional prominent bands and a second collection of isolates (SP133, Sp135, Xv18-OH, Xcv931, Xcv939 and Xv89) yielded a single additional band. REP-PCR differentiated Group B strains similar to BOX- and ERIC-PCR (Figure 4B). However, Xv56 yielded an additional band as compared to BV4-1 and BV6-1 (data not shown).

The combined data (BOX, ERIC and REP) appeared to provide a more detailed assessment of the chromosomal structure and strain diversity as compared to data generated by one primer set alone. Strain specific rep-PCR fingerprint patterns were

FIGURE 3. Agarose gel electrophoresis of fingerprint patterns obtained from genomic DNA from isolates of *Xanthomonas campestris* pv. vesicatoria Group A using primers corresponding to ERIC sequences. Other details are as outlined in the legend of Figure 1.

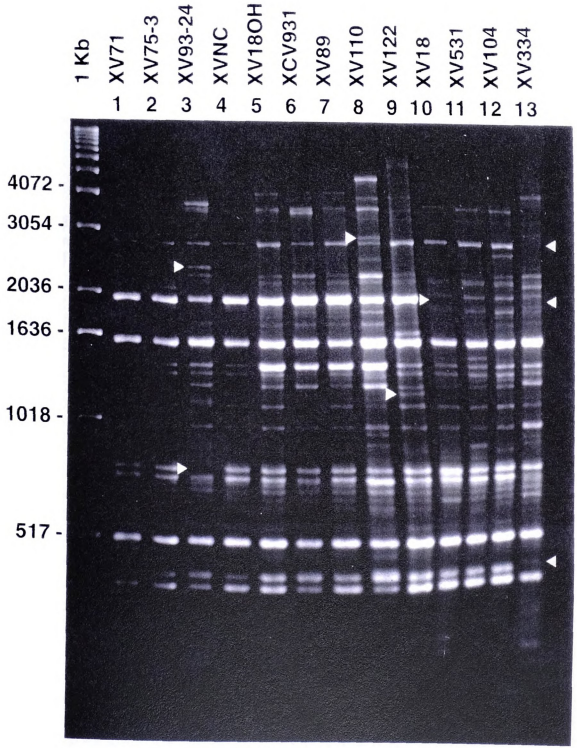

1 Kb
XV71
XV75-3
XV93-24
XVNC
XV18OH
XCV931
XV89
XV110
XV122
XV18
XV531
XV104
XV334
1 2 3 4 5 6 7 8 9 10 11 12 13
4072 -
3054 -
2036 -
1636 -
1018 -
517 -

FIGURE 4. Agarose gel electrophoresis of fingerprint patterns obtained from genomic DNA from isolates of *Xanthomonas campestris* pv. vesicatoria Group B using ERIC (A) and REP (B) primers. Other details are as outlined in the legend of Figure 1.

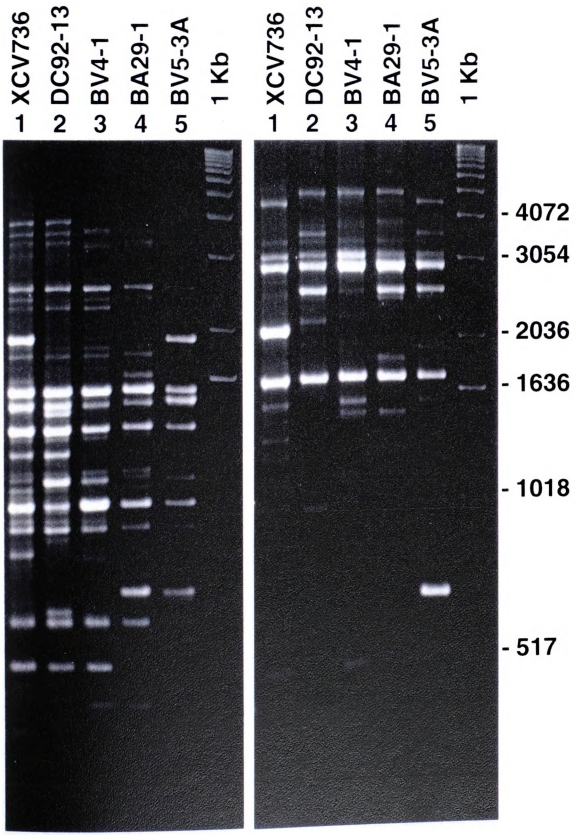
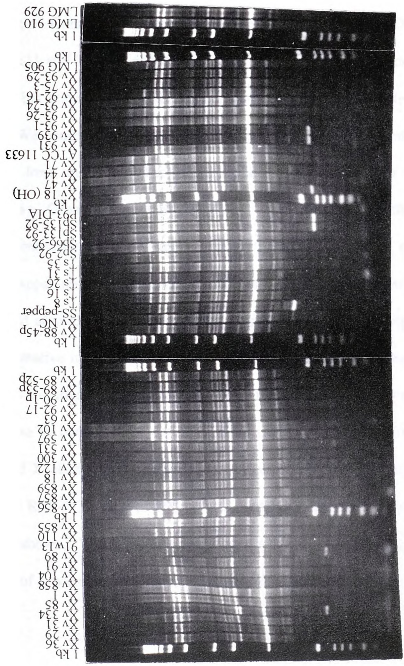

A) ERIC-PCR
B) REP-PCR
1 XCV736
2 DC92-13
3 BV4-1
4 BA29-1
5 BV5-3A
1 Kb
1 XCV736
2 DC92-13
3 BV4-1
4 BA29-1
5 BV5-3A
1 Kb
- 4072
- 3054
- 2036
- 1636
- 1018
- 517

FIGURE 5. Agarose gel electrophoresis of fingerprint patterns obtained from genomic DNA from isolates of *Xanthomonas campestris* pv. vesicatoria Group A using primers corresponding to REP sequences. Other details are as outlined in the legend of Figure 1.

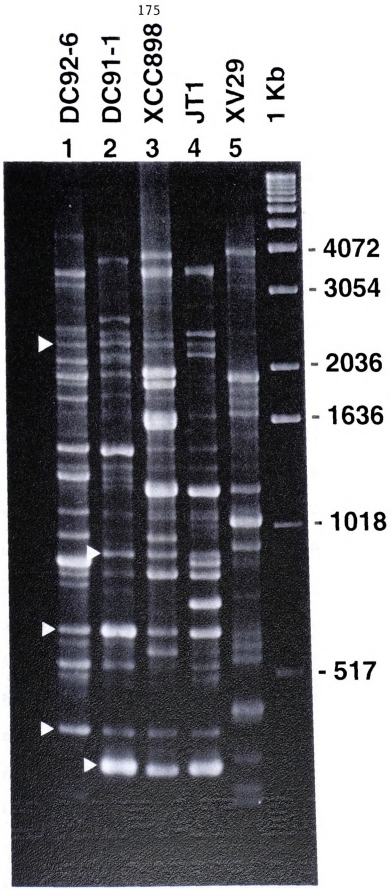

1 kb
Xv 36
Xv 29
Xv 31
Xv 334
Xv 85
Xv 1
Xv 858
Xv 104
Xv 91
Xv 89
91w13
Xv 110
Xv 855
1 kb
Xv 856
Xv 857
Xv 859
Xv 18
Xv 122
Xv 300
Xv 531
Xv 597
Xv 102
Xv 63
Xv 92-17
Xv 90-1p
Xv 89-53p
Xv 89-52p
1 kb
1 kb
Xv 88-45p
Xv NC
SS-pepper
Ts 8
Ts 16
Ts 26
Ts 31
Ts 35
Sp2-92
Sp66-92
Sp133-92
Sp135-92
P93-DIA
1 kb
Xv 18 (OH)
Xv 47
Xv 44
Xv 71
ATCC 11633
Xv 931
Xv 939
Xv 93-1
Xv 93-26
Xv 93-24
Xv 92-16
Xv 75-3
Xv 93-29
LMG 905
1 kb
1 kb
LMG 910
LMG 929

much more distinct within Group B. Group B appears to be comprised of a more heterogenous group of strains than Group A.

Genotype of Group D Strains. The isolates classified as Group D (Figure 1, lanes 4&5, 10&11 and 16&17) appeared to have several prominent bands of parallel mobility, but numerous additional bands were amplified to generate strain specific profiles. We compared the rep-PCR fingerprint profile of these strains to those generated from numerous other xanthomonad pathovars during our studies. We found that the two Group D strains shared several bands in common with isolates of *Xanthomonas campestris* pv. campestris (Xcc) (Figure 6). Several rep-PCR generated bands of apparently analogous DNA products between Group D and Xcc strains comigrated after BOX-PCR analysis (highlighted by arrowheads, Figure 6, lanes 1-4). A representative isolate of Xcv (Xv29, lane 5) did not appear to share more than 1 or 2 bands with Group D or Xcc strains. Three REP-PCR bands between 3 to 3.9 kb appeared to be common between the 2 Group D strains and two Xcc strains, ATCC 33913 and Xcc898 and also representative isolates of Xcv (data not shown). An additional REP-PCR band of 800 bp was common to the Group D and Xcc strains (data not shown). The BOX-PCR experiments, however, provided the strongest evidence of a possible close genetic relationship between the Group D and Xcc strains.

Reproducibility of fingerprints. The similar rep-PCR fingerprint profiles generated from isolates separated by a 40-50 year period attest to the reproducibility of fingerprints generated by REP-, ERIC- and BOX-PCR. In addition, the pathovar

FIGURE 6. Agarose gel electrophoresis of fingerprint patterns obtained from genomic DNA from isolates classified as *Xanthomonas campestris* pv. vesicatoria Group D (lanes 1&2) as compared to patterns generated from representative Group A isolates (lane 5) and isolates of *Xanthomonas campestris* pv. campestris (lanes 3&4) using BOX primers. Other details are as outlined in the legend of Figure 1.


DC92-6
DC91-1
XCC898
JT1
XV29
1 Kb
1
2
3
4
5
4072
3054
2036
1636
1018
517

reference strain was received as a culture or DNA from 3 independent sources (ATCC; J. Jones, Florida; and J. Swings, Belgium). Each primer set yielded identical profiles from DNA for each of the 3 samples. Likewise, duplicate samples of other strains were received over time and/or independently prepared and analyzed to yield identical fingerprints (data not shown). Dispensing cells directly into the PCR tubes from liquid or solid medium cultures also yielded fingerprint patterns identical to patterns generated from isolated DNA (data not shown). Finally, unique bands, such as those highlighted by arrowheads in Figure 2A, could be reproduced by independent rep-PCR experiments and independent analysis of aliquots of the same PCR mixtures on agarose gels.

PHENOTYPIC CHARACTERISTICS WITHIN GROUP A, B, C, AND D.

Because the fingerprint profiles between the four different groups were so distinct, we conducted several phenotypic tests to determine if specific phenotypes were associated with each group. Starch utilization, pectolytic activity and cellulolytic activity are common tests to differentiate Xcv from other pathogens and saprophytes in Georgia and Florida (Gitaitis et al. 1987). CKTM is a medium selective for the isolation of Xcv and is able to differentiate tomato and pepper strains (Sijam et al. 1991, 1992).

GROUP A Phenotype: Isolates classified within Group A were obtained from various parts of the world, were commonly isolated from tomato or pepper and were non-pectolytic (Table 1). Seventy- seven percent of Group A isolates were starch negative, 21% hydrolyzed starch weakly and one isolate (Xv334) was starch positive (Table 1).

Within Group A, a total of 18 and 35 isolates originated from tomato and

pepper, respectively (Table 1). All Group A isolates tested, except Xv93-24, formed a precipitate on CKTM medium. Although the biochemical and genetic basis for the differential reaction on CKTM is not known, 82% of strains isolated from pepper formed a pepper-type precipitate as described by Sijam et al. (1992). Fifty percent of the strains isolated from tomato did not form a distinct tomato-type precipitate. Five of these 9 isolates formed a pepper precipitate on CKTM medium and came from Ontario (Ts8,Ts16,Ts26,Ts31,Ts35). Each isolate was obtained from a different field but all in the same general geographic region (Southwest, Ontario) and in 1990 (Dr. Dhanvantari, personal communication).

GROUP B Phenotype: Isolates classified as group B also came from various parts of the world, including South America, New Zealand, Canada (ON), and the United States (MI & OK) (Table 1). All isolates evaluated and classified within Group B were able to hydrolyze starch, demonstrated pectolytic activity on CVP medium and with one exception (BA27-1) originated from tomato (Table 1).

Ninety one percent of the Group B strains did not form a halo on CKTM medium (Table 1). ICBB167 formed a very subtle clear ring within 3 days and BV7-3A and Xv10 had a light white halo after 4 and 6 days, respectively.

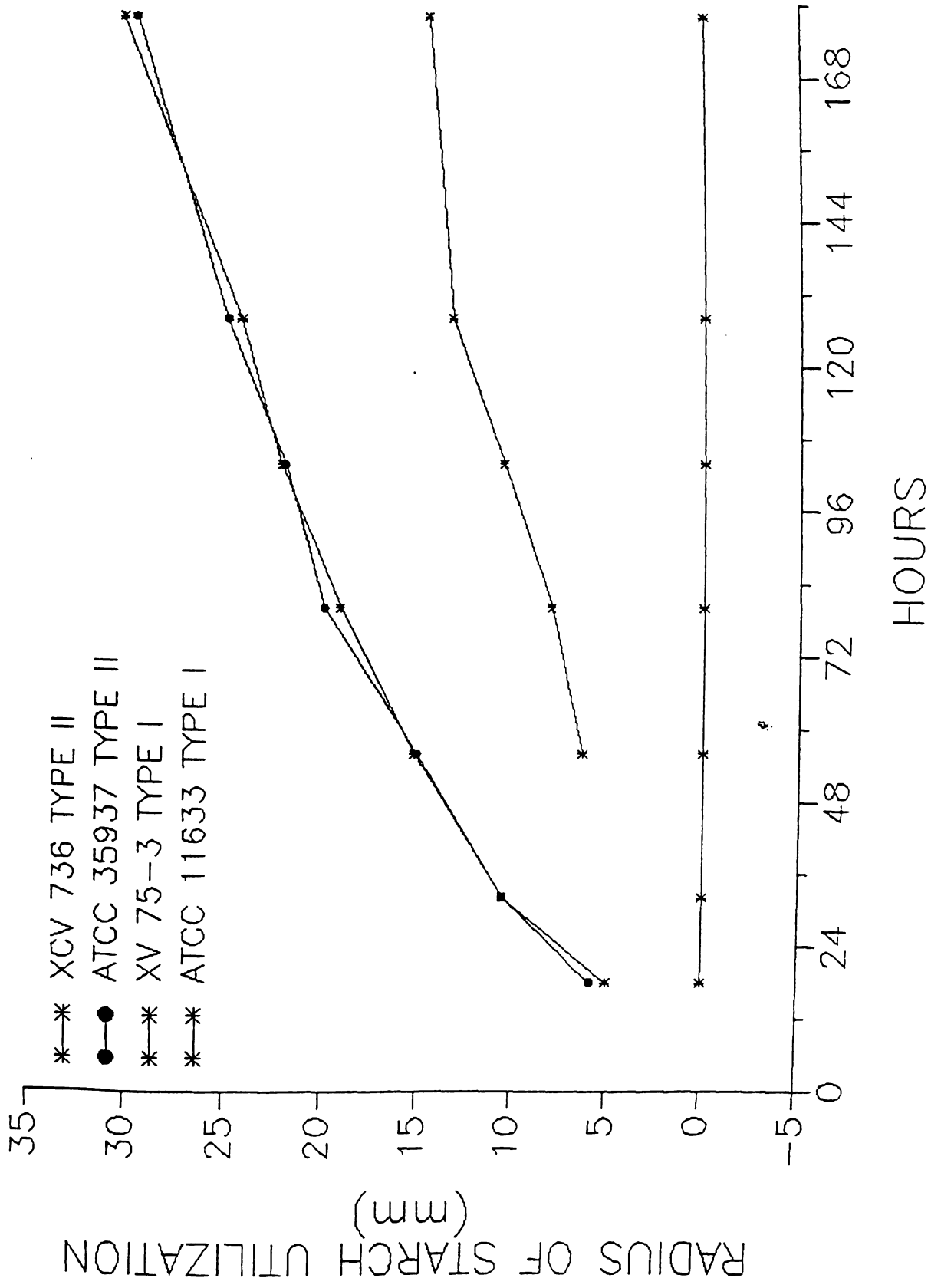
GROUP C and D Phenotype: Strain Xv441, the single isolate classified as Group C, originated from tomato and was negative for both amylolytic and pectolytic activity (Table 1). Xv441 formed a subtle ring on CKTM similar to strain ICBB167 of Group B (Table 1). The two Group D isolates, DC91-1 and DC92-6 from Ontario, originated from tomato greenhouse transplants (D. Cuppels, personal communication), were starch and pectolytic positive and formed a distinct tomato-type halo on CKTM media (Table 1).

All Xcv strains tested demonstrated cellulolytic activity (data not shown). Isolates of *Xanthomonas campestris* pv. campestris were CMC positive, positive for starch hydrolysis and pectolytic activity and formed a tomato-type precipitate or no precipitate on CKTM (Table 1). Other pathovars of Xc were CMC positive and were negative or positive for starch hydrolysis and pectolytic activity (Table 1). *Pseudomonas* isolates effectively functioned as controls and scored negatively on CVP, starch medium (Table 1) and CMC.

Quantitative assessment of starch hydrolysis: Several isolates were found to hydrolyze starch weakly (Table 1) and this is a common description for strains classified as *Xanthomonas campestris* pv. vesicatoria (Dye 1962; Dye et al. 1964; Gardner and Kendrick 1921). Therefore an assay was performed to determine the quantitative differences between strains that hydrolyze starch weakly as compared to those with strong hydrolytic activity. Isolate ATCC 35937 (pathovar reference strain) and Xcv 736 demonstrated identical amylolytic activity (Figure 7). Both isolates formed clear zones within 18 hours and the zones expanded at a rate of 0.12 mm per hr between hrs 56 to 180 (Figure 7). A representative starch negative isolate, ATCC 11633, did not form a zone even after 7 days. Xv75-3, a weak hydrolyzer of starch, did not form a distinct zone until after 56 hrs and the subsequent rate of zone expansion was 0.07 mm per hr thereafter.

Distribution of Races. Each described known race sensu Minsavage et al. (1990), including tomato race 1 (T1 of the XcvT group), pepper race 1 (of the XcvP group), pepper race 2 (of the XcvPT group) and pepper race 3 (of the XcvPT group) had

FIGURE 7: Increase (mm) in the radius of cleared zones indicating starch hydrolysis by selected isolates of *Xanthomonas campestris* pv. vesicatoria.


RADIUS OF STARCH UTILIZATION
(mm)
35
30
25
20
15
10
5
0
-5
0
24
48
72
96
120
144
168
HOURS
XCV 736 TYPE II
ATCC 35937 TYPE II
XV 75-3 TYPE I
ATCC 11633 TYPE I

genomic rep-PCR fingerprints characteristic of Group A (Figures 2A and 3A). Race designation of isolates within Group A could not be correlated to total chromosomal fingerprint patterns as determined by rep-PCR. Isolate Xv441 from the Caribbean Islands has been classified as race 1 (personal communication, H. Bouzar and J. Jones, Gulf Coast Research Center, Florida) and was the single member of Group C.

DISCUSSION

The precise function of REP, BOX and ERIC sequences is not known (Lupski and Weinstock 1992) but these repetitive sequences were exploited via PCR to rapidly assess the genetic diversity of strains classified as *Xanthomonas campestris* pv. vesicatoria. Based on this study, strains classified as *Xanthomonas campestris* pv. vesicatoria clearly fell into 4 completely different groups, designated A, B, C and D, Isolates appear to be highly homogenous within Group A and isolates within Group B are more genetically diverse.

We noted polymorphisms among Group A, B and D isolates by REP- BOX- and/or ERIC-PCR. Polymorphisms were simple (with differences limited to 1 to 3 DNA bands with any given primer set) within Group A, with the exception of Xv334. This isolate was shown to be polymorphic using all 3 primer sets. Xv334 was also physiologically atypical having starch hydrolytic activity. In contrast, in our limited sample of Group B, 5 distinct patterns, or lineages, could be elucidated by each primer set. Stall et al. (1993) have also concluded that Group B appears to comprise a more heterogenous collection of strains as compared to Group A.

Despite the heterogeneity of patterns within Group B, it does not appear to be

possible to follow a specific strain in epidemiological studies using the rep-PCR. For example, Xcv859 was isolated in 1 of 112 plots during a large integrated disease management study in Michigan during the 1991 season (Chapter 2). In 1992, Xcv736 was isolated from the exact same plot and yielded a fingerprint identical to Xcv859, suggesting the strain had overwintered under the minimum tillage conditions employed. However, isolates from Ontario (TS1) and Oklahoma (Xv10 and Xv15) also had identical fingerprint patterns (Figure 2B and 3). Although unlikely in this particular field study, the possibility that Xcv736 was reintroduced as seed-borne inoculum could not be ruled out.

Xcv has been described as a pathovar comprised of diverse strains (Doolittle and Crossan 1959, Dye 1962, Dye et al. 1964, Jones et al. 1993a, Minsavage et al. 1990, Klement 1959, Sutic 1959, Vauterin et al. 1990, 1991, Wang et al. 1990, Whalen et al. 1988). This report highlights the complexity of the observed diversity based on the ability of different isolates to hydrolyse starch, their pectolytic activity, reaction on CKTM medium, host of origin and pathogenicity. However, in this study we have been able to categorize such diversity within a useful genotypic framework as determined by rep-PCR. For example, Xcv has been described with the ability (Gardner and Kendrick 1921), inability (Gitaitis et al. 1987) and variable ability (Burkholder and Li 1941; Dye et al. 1964) to hydrolyse starch and demonstrate pectolytic activity (Beaulieu et al. 1991). Our work demonstrates that amylolytic and pectolytic activities are highly associated with specific groups. Over 96% of the isolates evaluated in this study were classified as Group A or Group B and the isolates in each group were predominantly amylolytic/pectolytic minus or amylolytic/pectolytic plus, respectively.

Vauterin et al. (1990, 1991) have classified Xcv strains into two sub-pathovar categories, subgroup A and B, based on fatty acid profile analysis, DNA homology studies and SDS-PAGE Electrophoresis. Likewise, Jones et al. (1993) and Stall et al. (1993) have classified Xcv strains at the subpathovar level as Strain A and Strain B based on polyphasic criteria. We obtained representative samples from both research groups and learned that subgroup A and subgroup B is synonymous with Strain A and Strain B, respectively. Prior to a comparative analysis of the alternative subpathovar classification systems, we had classified the various groups discerned by rep-PCR as group I, II, III and IV (Int. Congress Plant Pathology, Montreal, 1993). However, to avoid confusion and because current use of A and B is in agreement, we also used alphabetical letters in this chapter to name the groups.

Harmonizing the two subpathovar groupings allowed a more comprehensive understanding of the diverse attributes associated with group A and B. For example, Vauterin et al. (1990, 1991) have shown A and B strains belong to distinct DNA homology groups and have different SDS-PAGE profiles. Isolate LMG 920 and the pathovar reference strain (ATCC 35937) have been classified into separate subgroups based on SDS-PAGE profiles (Vauterin et al. 1991) but could not be distinguished by rep-PCR (Figure 2, bottom).

Stall et al. (1993) have demonstrated that Group A and Group B can also be differentiated by monoclonal antibodies and restriction endonuclease analysis among other methods tested. They also corroborate our observations of pectolytic activity and starch hydrolysis associated within Group B but not Group A.

The REP-, BOX-, and ERIC-PCR amplified identical profiles from genomic DNA of the Group B strains BA27-1 and BA29-1 and this profile differed from BV5-

3A and Xv56. Stall et al. (1993) have also used these isolates in their study and showed monoclonal antibodies could distinguished BA27-1/BA 29-1 as compared to BV5-3A and Xv56. Based on restriction digest analysis Stall et al. (1993) also noted that BV27-1 and BV29-1 have a very small genetic distance whereas Xv56 and BV5-3A have a greater genetic distance, further corroborating the results of this study.

The fact that strains classified as Group A and B are so dissimilar suggests that pathogenicity for tomatoes occurred through convergent evolution and that the population structure of Xcv is polyphyletic. The relative contribution of recombinational convergence or mutational convergence (Selander and Musser 1990) is not known. If recombinational convergence presides, then limited host-specific virulence genes (Swarup et al. 1992; Waney et al. 1991) may associate with diverse genomic backgrounds to give rise to new genotypes pathogenic for tomato and/or pepper but with an unaltered host range. For example, Group D and Group A isolates may have the same host-specific gene in a different genetic background. In such cases, the possibility of developing durable genetic resistance appears to hold more promise than if pathogenicity occurs through mutational convergence i.e. perhaps through the mutation of an avirulence factor (Kearney et al. 1988). In the latter case, rates of mutation and selective pressure may always favor parasitism.

The importance of Group C and D genotypes and the presence of other genotypes pathogenic to tomato and/or pepper is not known. The single group C strain (Xv441) was discovered in the Caribbean region and has been noted to be unique based on polyphasic phenotypic experiments (H. Bouzar and J.B. Jones, University of Florida, personal communication). The Group D isolate DC91-1 is a highly virulent and destructive pathogen based on our pathogenicity tests (data not shown) and

economic damage observed in a commercial greenhouse (D. Cuppels, personal communication, Agriculture Canada, London, Ontario). DC92-6 was recovered from tomato seedlings in the greenhouse, shares numerous comigrating bands as DC91-1 but does not appear to be highly virulent to tomato (data not shown). Based on common bands between these Group D strains and isolates representative of *Xanthomonas campestris* pv. campestris (Xcc), our data suggests the Group D strains may have originated from or have a common ancestry as Xcc. Additional sampling and evaluation may in fact reveal other genotypes able to incite bacterial spot of tomato. Assessing and monitoring clonal population structures may provide a means for determining the potential of new genotypes to emerge and become prominent through periodic selection and extinction (Levin 1981) forces and provide some insight into the evolutionary dynamics of plant pathogenesis (Selander and Musser 1990). The detection of these genotypically distinct strains (DC91-1 and DC92-6) invites numerous questions on the origin of these strains, the genetic differences between the highly virulent strain (DC91-1) and the less virulent strain (DC92-6), and the potential of the highly virulent strain to become a predominant clone.

The importance of discerning genetic diversity is highlighted by our findings that Group B strains comprise an important component of the tomato spot complex in the northcentral production region. During the initial phase of our study, most strains we evaluated belonged to Group A. Local Michigan isolates obtained from infected tomatoes yielded a totally dissimilar fingerprint profile via rep-PCR when compared to Group A strains and we thought we may have isolated saprophytic xanthomonads (Gitaitis et al. 1987). However, when we obtained the pathovar reference strain (ATCC 35937) and numerous other isolates from Ontario, the latter new isolates had a similar

fingerprint profile as our Michigan isolates and prompted us to determine their amylolytic and pectolytic activity. Our results indicate Group B strains are more widely distributed than previously thought. Most work with Xcv has been conducted in Florida and Georgia where the majority of isolates are diagnostically unable to hydrolyze starch and are non-pectolytic (Beaulieu et al. 1991; Gitaitis et al. 1987; Stall et al. 1993). Beaulieu et al. (1991) concluded pectolytic activity was correlated with the geographical origin of isolation, since 90% of isolates from Argentina had pectolytic activity (including isolates used in our study such as Xv 56, BV5-3a, and BA27-1, i.e. Group B) compared to 0.003% from the United States. However, strains obtained from numerous epidemics in Ontario (DC92-13, DC92-21, DC92-23, TS1, CC164#3 and CC195#1), Michigan (Xcv859, 736, 981, 982), Indiana (ATCC 11551) and Oklahoma (Xv 10 and Xv 15), all belong to Group B.

Although it is difficult to ascertain, low levels of Xcv detected on tomato seed as compared to pepper seed may be a function of detection protocols used. In the future, protocols should be designed to detect, at the minimum, Group A and B isolates. Likewise, plant breeding programs will need to include representative strains of each group. Diagnostic and breeding programs need to be integrated into the overall tomato production system and are knowledge based and dependent on the tomato industry as a whole, as compared to field level integrated management strategies. Intensive research and development is required to ensure Xcv is not a problem on farms. Once Xcv becomes established in a field, there is little a grower can do to control the epidemic in susceptible cultivars.

In conclusion, elucidating the genotypic structure in natural populations of strains classified as Xcv provided a framework for mapping the diversity of

phenotypic traits including virulence and pathogenicity. Knowledge of the population structure of Xcv should aid in the selection of representative isolates for taxonomic analyses, for evolutionary, ecological, epidemiological studies, and for devising integrated disease management of bacterial spot of tomato such as diagnostic, detection and plant breeding programs.

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CHAPTER V

CONCLUSIONS AND FUTURE RESEARCH

CONCLUSIONS

Over 70 to 100 years of research, embracing hundreds of independent studies, document the epidemiology, etiology, and pathology of fungi and bacteria pathogenic to tomato. An historical emphasis on chemical based disease control has left a void in scientific and lay literature concerning the potential and implementation of alternative forms of disease control. The relatively cheap and effective use of chemicals has enabled the tomato industry to make significant advancements in productivity (yield per hectare) without relying on genetic resistance, cultural practices, or an understanding of pathogen diversity for disease control. However, in the face of declining chemical options, alternative disease management options are urgently needed. In the case of genetic resistance, the limitation is not simply a lack of useful germplasm, but is also likely due to a lack of emphasis. Likewise, cultural practices must now be considered more seriously as a component of integrated disease management strategies.

The knowledge base is limited concerning the effects and potential of rotation (length and crops), cover and green manure crops, reduced tillage, and other agronomic activities on management of disease in tomato production systems. Likewise, limited information is available concerning the genetic diversity of tomato pathogens. Advancements in cultural and bio-technologies are becoming available and the balance of genetic, cultural and biotechnological inputs for integrated disease

management will be influenced by the pathosystem.

Fundamentally, as highlighted in this thesis, the most effective integration of disease management strategies is based on the divergent mechanisms by which the fungi and bacteria become epidemiological problems. The fungi are indigenous to the region and appear to reside in the agro-ecosystem at inoculum levels high enough to limit fruit quality even in fields with no history of tomato. Ultimately, disease management of the fungal fruit-foliar pathosystem is dependent on farm level decisions and inputs. Based on this thesis, it is possible to minimize the number fungicide applications required for the control of early blight, anthracnose, and soil rot of fresh market and processing tomato through the use of tillage, a green manure crop, and weather timed fungicide sprays even within a biennial (tomato/cucumber) conservation tillage production system. The benefits of conservation tillage, cover crops and reduced fungicide input can be integrated without compromising fruit quality and yield.

The bacteria are generally considered introduced problems and grower attempts to control epidemics in susceptible cultivars have marginal impacts, especially during climatic conditions that favor disease progress. One of the most prominent Michigan growers of tomato plug transplants testified that the bacterial diseases appear to become a problem despite any and all efforts on his part to try and limit them (M. Hausbeck, personal communication). "Prevention is the key" (Goode and Sasser 1980) to integrated management and can only come about through industry dependent activity such as private and public research and certification protocols. Bacterial canker, bacterial speck and bacterial spot were encountered at economic levels in plots of the field experiments outlined in this thesis (Appendix B) but the spot organism

was chosen as a model system for assessing genotypic diversity. Rep-PCR applied to Xcv uncovered a population diversity that has direct consequence for disease management. Starch positive and/or pectolytic strains comprise an important component of the spot problem in the north-central production region. Attempts to develop genetic resistance, assay seed lots for seed-borne inoculum, and the development of diagnostic/identification protocols must consider such diversity. This requires high inputs in terms of research and development before "the seed is planted".

FUTURE RESEARCH

Integrated disease management of the fungal disease pathosystem is likely to be advanced in several ways. Considerable benefit was obtained with zone tillage (ZT) in plots with no recent history of tomato. The length of rotation and type of crop may impact initial inoculum and impact the ZT advantage each year. Although rotation is routinely recommended, limited quantitative information appears to be available with regard to inoculum levels and subsequent disease progress. Long term studies incorporating various rotation treatments may provide a knowledge base for future recommendations.

Management of the fungal pathosystem is likely to benefit most from genetic resistance. Resistance to early blight, anthracnose and soil rot is known (Barksdale 1974; Barksdale and Stoner 1981; Gardner 1988) but is generally polygenic and difficult to transfer into commercial cultivars. However, such forms of resistance (i.e. horizontal) may be durable as opposed to resistance based on single genes (i.e. vertical). If horizontal-type resistance can be successfully incorporated into commercial lines, fungicide input can be further reduced. For example, O'Leary (1985) has shown resistance to early blight in Dr. Gardner's advanced lines is governed by the interaction of infection efficiency, lesion area, latent period, and sporulation capacity. This rate-reducing resistance can be combined with a reduced-sprays program to achieve control (O'Leary 1985). Likewise, Barksdale and Stoner (1981) demonstrated resistance to anthracnose provided control of fruit rot equivalent to 3 - 7 fungicide applications.

Biotechnological advancements also hold promise for enhanced disease control. Anti-sense polygalacturonase expression in transformed tomato fruit limits fruit rot (Kramer et al. 1992). Chitinase (Broglie et al. 1990) and other pathogenesis related

proteins can also be genetically engineered into tomato. Accumulation of chitinase and other anti-fungal proteins has been associated with resistance to *Alternaria solani* (Lawrence and Tuzun 1992). This form of resistance is likely to be rate-reducing and research would need to elucidate how engineered resistance can best be deployed so it remains durable. Stewardship of non-renewable resources (i.e. effective genetic resistance, natural or otherwise) may benefit from an understanding of the population diversity of the fungal pathogen. Likewise, resistance may remain durable if deployed within integrated disease management systems. Research is required to assess the diversity of *A. solani*, *R. solani*, and important *Colletotrichum* species.

Biological control holds some promise but considerable basic research is required to understand the dynamics of site specific deployment and mechanisms by which introduced organisms provide control.

Advances in integrated disease management of bacterial disease of tomato may also be accomplished with genetic resistance and biotechnological advances. To date, acceptable levels of genetic resistance in tomato has not proven durable (Lawton and MacNeill 1986, Wang et al. 1990). Considerable basic research is required to understand the interaction between the bacteria and host. Identifiable resistance in the host is often governed by a single dominant gene that specifically interacts with a dominant gene for avirulence in the bacteria. However, avirulence genes may be gratuitous (Gabriel 1989) and simply associated with genes for pathogenicity that govern basic compatibility (Heath 1991). Loss of an avirulence gene in such cases may be of no ecological importance to the bacteria other than circumventing a hypersensitive host- defence response. Alternatively, genes that elicite a defence response may be important as a component of virulence. The REP-, BOX-, and ERIC-

PCR appear to provide a survey of the chromosomal structure of bacteria. Specific rep-PCR fingerprint profiles may be associated with host specific virulence genes (Waney et al. 1991) or species specific genes (Heath 1991) that function in a positive manner to incite disease on specific hosts. A fundamental understanding of the basic mechanism by which the bacteria cause disease will likely be arrived at by first elucidating the population structure of the pathogen. Once classified into "lineages" (Levy et al. 1991; Leach et al. 1992), experiments can be designed to elucidate the mechanisms of basic compatibility and this knowledge may be useful to develop resistance. Fundamental research is also required to determine how host resistance (i.e. basic resistance sensu Heath) may impact basic compatibility of the pathogen.

Characterization of the population diversity of Cmm and Pst should also be future research. Ability to describe variability of each pathogen may be of practical use for the deployment of host resistance (Levy et al. 1991; Leach et al. 1992).

Detection and identification protocols also need to be advanced and will likely have the largest short-term impact. The REP-, BOX-, and ERIC-PCR can provide a framework for elucidating important sub-populations that must be detected. Serological or DNA hybridization based detection systems may then be devised. Diagnostic probes specific for Pst (Cuppels et al. 1990) and Cmm (Thompson et al. 1989) have been developed but additional research is required to integrate their use.

Management of early blight, anthracnose, soil rot and bacterial diseases of tomato is possible with current levels of technology and knowledge. However, additional research is required to expand and integrate this technology and knowledge, and formulate advanced integrated disease management strategies in tomato production systems.

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APPENDIX A

Table A1: Mean squares from analysis of variance for yield and fruit quality of fresh market tomato (FMT) in 1992 using 4 replications.

Source of Variability	df	FMT FRUIT QUALITY AND YIELD (MT/HA)[x]				
		LARGE #1	%LRG #1	MED #1	#2	CULL
Rep.	3	31 NS	280 NS	14 NS	1965 *	11 NS
Rotation (R)	1	979 *	474 NS	0.5 NS	85 NS	4 NS
Error a	3	105	86	11	197	44
Tillage (T)	1	916 NS	225 NS	31 *	671 *	20 NS
R x T	1	671 NS	>1 NS	43 **	794 *	278 **
Error b	6	231 NS	191	3	81	16 NS
Fung.(F)	6	110 *	80 *	3 NS	137 *	17 NS
R x F	6	5 NS	26 NS	2 NS	12 NS	25 NS
T x F	6	61 NS	50 NS	4 NS	18 NS	6 NS
R x T x F	6	56 NS	32 NS	4 NS	42 NS	14 NS
Error c	72	45	32	4	50	12

*, **, *** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for large #1, % large #1, medium #1, #2 and culls.

TABLE A2: Effect of rotation and tillage on marketable fruit of fresh market tomato in 1992 using 4 replications.

TILLAGE	LARGE NO.1		MT/Ha		
	MT/Ha	%	MEDIUM NO.1	NO.2	CULL
NO ROTATION	19.6	29	8.5	22.5	17.2
WITH ROTATION	25.5	33	8.7	24.3	17.6
P VALUE	0.055	0.10	<0.30	<0.30	<0.30
CT	19.7	29	8.1	20.9	17.0
ZT	25.4	32	9.2	25.8	17.8
P VALUE	0.09	<0.30	0.02[y]	0.03[z]	0.29

[y] interaction is significant (R- x CT=8.65; R+ x CT=7.56; R- x ZT=8.47; R+ x ZT=9.84; LSD=1.17 P=0.05).
[z] interaction is significant (R- x CT=22.7; R+ x CT=19.2; R- x ZT=22.3; R+ x ZT=29.4; LSD=5.89 P=0.05).

TABLE A3. Effect of fungicide treatment on marketable yield of fresh market tomato in 1992 using 4 replications.

FUNGICIDE TREATMENT	LARGE NO.1		MT/Ha		
	MT/Ha	%	MEDIUM NO.1	NO.2	CULL
WEEKLY	26.2	34.5	8.5	25.0	15.7
DSV 25H	24.2	31.9	8.6	23.7	17.3
NO SPRAY	18.5	28.4	8.1	17.6	17.7
P VALUE	0.03	0.03	<0.30	0.02	0.22
LSD;P=.05	4.7	4.0		5.0	

TABLE A4: Mean squares from analysis of variance for yield and fruit quality of processing market tomato (PRT) in 1992 using 4 replications.

Source of Variability	df	PRT FRUIT QUALITY AND YIELD (MT/HA)[x]			
		RIPE	GREEN	CULLS	% RED
Rep.	3	156 NS	3394 *	13 NS	1980 NS
Rotation (R)	1	36 NS	34 NS	0.4 NS	505 NS
Error a	3	140	192	12	590
Tillage (T)	1	773 ***	1564 **	30 *	580 NS
R x T	1	722 ***	407 NS	22 *	1054 *
Error b	6	16	124	3	124
Fungicide (F)	6	41 NS	635***	31 ***	394 ***
R x F	6	6 NS	43 NS	2 NS	49 NS
T x F	6	16 NS	18 NS	12 NS	55 NS
R x T x F	6	17 NS	104 NS	16 NS	30 NS
Error c	72	23	79	3	85

*,**,*** F-test significant at P = 0.05, P = 0.01 or P = 0.001, respectively.
NS, non-significant.

[x] Fruit were sorted for color (ripe or green) and quality (marketable fruit or culls).

TABLE A5: Effect of tillage on marketable yield of processing tomato cv. Heinz 8780 in 1992 using 4 replications.

TILLAGE	MT/Ha			% RED
	RIPE	GREEN	CULLS	
NO ROTATION	12.1	24.1	2.9	32
WITH ROTATION	10.9	25.2	2.7	28
P VALUE	<0.30	<0.30	<0.30	<0.30
CT	8.9	20.9	2.3	27
ZT	14.1	28.4	3.3	32
P VALUE	0.0004[y]	0.01	<0.02[z]	0.07

[y] interaction is significant (R- x CT=12.0; R+ x CT=5.8; R- x ZT=12.1; R+ x ZT=16.1; LSD=2.54 P=0.05).
[z] interaction is significant (R- x CT=2.8; R+ x CT=1.8; R- x ZT=2.9; R+ x ZT=3.7; LSD=0.98 P=0.05).

TABLE A6. Effect of fungicide treatment on marketable yield of processing tomato in 1992 using 4 replications.

FUNGICIDE TREATMENT	MT/Ha			% RED
	RIPE	GREEN	CULLS	
WEEKLY	9.7	33.5	1.1	22.0
DSV 25H	9.7	28.2	1.3	24.0
NO SPRAY	10.8	13.3	4.9	35.8
P VALUE	0.12	>0.001	>0.001[y]	>0.001
LSD;P=0.05		6.3	1.3	6.5

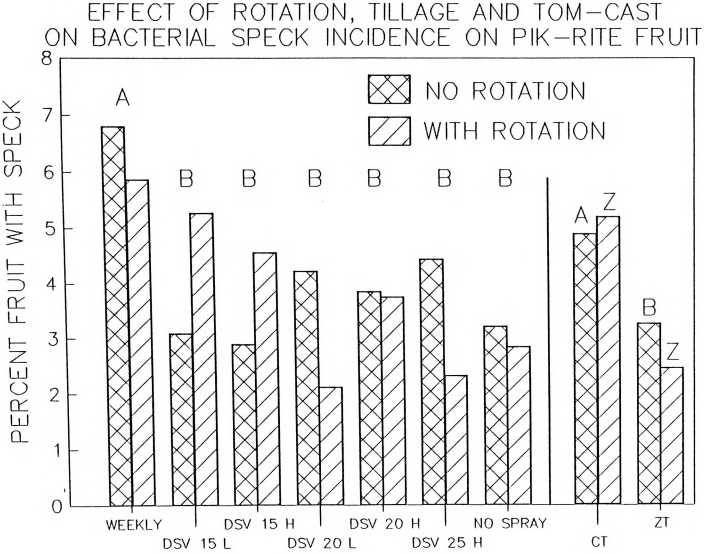
[y] The fungicide x tillage and fungicide x tillage x rotation interactions were significant.

APPENDIX B

EFFECT OF TILLAGE AND FUNGICIDE ON THE INCIDENCE OF BACTERIAL SPECK

Fresh market tomato fruit were harvested weekly in 1992 and fruit with bacterial speck symptoms was weighed (Chapter 2). Preliminary analysis of data indicate weekly applications of fungicide resulted in elevated levels of speck, caused by *Pseudomonas syringae* pv. tomato.

Zone tillage in non-rotation plots reduced speck incidence. Thus, cultural practices also have some influence on the populations of bacteria able to infect tomato.

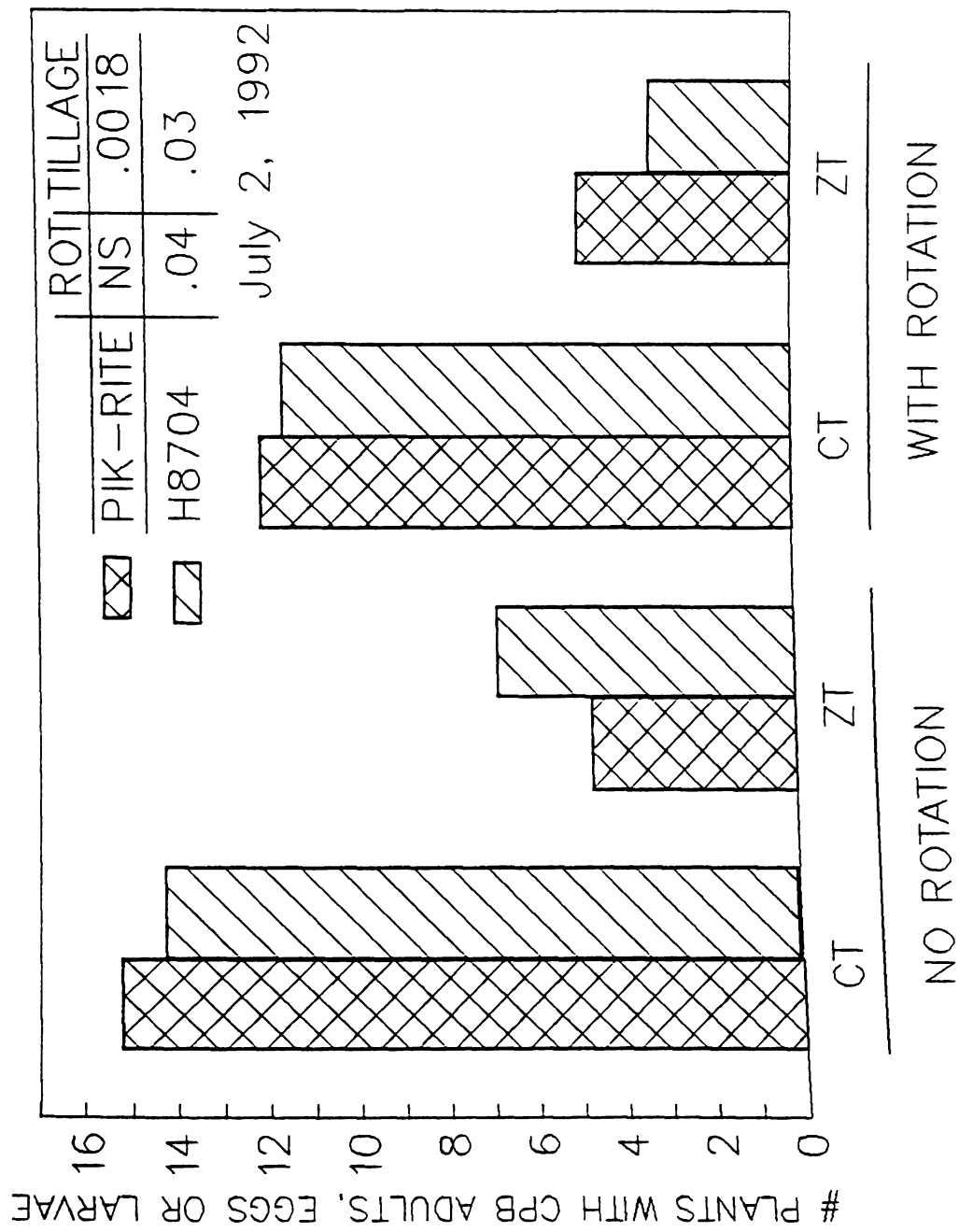

EFFECT OF ROTATION, TILLAGE AND TOM-CAST
ON BACTERIAL SPECK INCIDENCE ON PIK-RITE FRUIT
PERCENT FRUIT WITH SPECK
0
1
2
3
4
5
6
7
8
NO ROTATION
WITH ROTATION
A
B
B
B
B
B
B
A
Z
B
Z
WEEKLY
DSV 15 L
DSV 15 H
DSV 20 L
DSV 20 H
DSV 25 H
NO SPRAY
CT
ZT

APPENDIX B CONTINUED

EFFECT OF TILLAGE ON THE EARLY INFESTATION OF PLOTS BY THE COLORADO POTATO BETTLE

Total incidence of Colorado potato beetle was monitored early in the 1991 and 1992 seasons. In 1992, rotation alone did not seem to impact the number of fresh market tomato plants with CPB but reduced the incidence on processing plants. In contrast, zone tillage dramatically reduced CPB counts on all tomato plants. Excessive rye residues on the soil surface may disrupt the CPB from locating its host.

CPB


ROT TILLAGE
PIK-RITE NS .0018
H8704 .04 .03
July 2, 1992
PLANTS WITH CPB ADULTS, EGGS OR LARVAE
0
2
4
6
8
10
12
14
16
CT
ZT
NO ROTATION
CT
ZT
WITH ROTATION

APPENDIX C

DIRECT ANALYSIS OF BACTERIA ON MEDIA AND IN PLANT LESIONS

PURPOSE: To determine if fingerprint patterns can be generated using whole cells collected from media and from lesions on plant tissue to identify unknown isolates.

MATERIALS AND METHODS: *Clavibacter michiganensis* subsp. *michiganensis* (CMM 936), *Xanthomonas campestris* pv. vesicatoria (Xcv 982, 736), *Pseudomonas syringae* pv. tomato (PST 915), and unknown cultures were initiated on King's B (KB), nutrient yeast dextrose agar (NYDA), SCM2-t (selective for CMM), or Tween B (selective for XCV). After 7 days of growth, a low number of cells were collected and placed directly into epindorph tubes containing reagents for the polymerase chain reaction and primers corresponding to BOXA. Samples were subject to conditions outlined in Chapter 3.

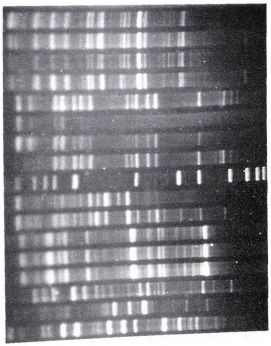
Samples of tomato from a commercial field with a presumptive bacterial epidemic was brought to the lab. Lesions were cut from fruit and a DNA miniprep was performed. One ul of extract was subject to PCR as described above.

DNA extracted from isolates proven to be true pathogens by Kochs postulates were used as positive controls.

RESULTS: CMM, XCV and PST cells collected from media yielded DNA fingerprints identical or similar to control DNA when subject to BOX-PCR. XCV collected from NYDA did not yield an acceptable pattern possibly due to high levels of polysaccharides on this rich medium. Extract from unknown lesions generated fingerprint patterns similar to XCV control DNA. Unknown samples isolated from an unknown spot on tomato fruit and collected from single colonies on NYDA generated unique fingerprint patterns as compared CMM, XCV and PST and could not be identified.

DISCUSSION and CONCLUSIONS: Cells of bacteria on selective or non-selective media can be rapidly identified using BOX-PCR. Our experience has been that colonies of CMM, XCV and PST when isolated from symptomatic plants appear on selective or non-selective media within 4, 2 to 4, and 2 days, respectively. Taken together, these data suggest rapid non-presumptive diagnosis of bacterial colonies can be accomplished for Gram positive and Gram negative phytopathogenic bacteria if control patterns are available for comparative analysis. Identification of bacteria directly from plant lesions is also possible but contaminating bacteria appear to alter patterns such that the fingerprint profiles are not identical to control DNA. This technique holds considerable possibilities for routine and rapid identification of unknown isolates.

I thank Nancy Fichter for conducting this and other similar experiments.



CMM 936 DIRECT KB
CMM 936 DIRECT NYDA
CMM 936 DIRECT SCM2-t
CMM 936 DNA
XCV 982 DIRECT KB
XCV 982 DIRECT NYDA
XCV 982 TWEEN B
XCV 736 DNA
1 kb LADDER
UNKNOWN DIRECT FROM LESION
UNKNOWN DIRECT FROM LESION
PST 915 DIRECT KB
PST 915 DIRECT NYDA
PST 915 DNA
UNKNOWN
UNKNOWN
UNKNOWN

APPENDIX D

SPECIFIC GENOMIC FINGERPRINTS OF PHYTOPATHOGENIC *XANTHOMONAS* AND *PSEUDOMONAS* PATHOVARS AND STRAINS GENERATED WITH REPETITIVE SEQUENCES AND PCR.

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Specific Genomic Fingerprints of Phytopathogenic *Xanthomonas* and *Pseudomonas* Pathovars and Strains Generated with Repetitive Sequences and PCR

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DNA primers corresponding to conserved motifs in bacterial repetitive (REP, ERIC, and BOX) elements and PCR were used to show that REP-, ERIC-, and BOX-like DNA sequences are widely distributed in phytopathogenic *Xanthomonas* and *Pseudomonas* strains. REP-, ERIC-, and BOX-PCR (collectively known as rep-PCR) were used to generate genomic fingerprints of a variety of *Xanthomonas* and *Pseudomonas* isolates and to identify pathovars and strains that were previously not distinguishable by other classification methods. Analogous rep-PCR-derived genomic fingerprints were generated from purified genomic DNA, colonies on agar plates, liquid cultures, and directly from lesions on infected plants. REP-, ERIC-, and BOX-PCR-generated fingerprints of specific *Xanthomonas* and *Pseudomonas* strains were found to yield similar conclusions with regard to the identity of and relationship between these strains. This suggests that the distribution of REP-, ERIC-, and BOX-like sequences in these strains is a reflection of their genomic structure. Thus, the rep-PCR technique appears to be a rapid, simple, and reproducible method to identify and classify *Xanthomonas* and *Pseudomonas* strains, and it may be a useful diagnostic tool for these important plant pathogens.

Plant pathologists are faced with the important challenge to discern plant-pathogenic variants within the species *Xanthomonas campestris* (Pammel 1895) Dowson 1939, *Xanthomonas oryzae* ex Ishiyama 1922, and *Pseudomonas syringae* van Hall 1902. These species are currently subdivided at the infraspecific level into 143, 2, and 45 pathovars, respectively (11, 52, 69, 70). Pathovars within each species cannot be reliably distinguished by their cellular metabolism or other phenotypic characteristics (10, 43, 54, 55). Therefore, they are classified on the basis of their distinctive pathogenicity to one or more host plants (69). Unfortunately, identification based on pathogenicity tests can be inconclusive and open to alternative interpretations (15, 17, 37). Several attempts have been made to classify pathovars and strains by using alternative features of the pathogen. Serologic testing (3, 4), fatty acid profiling (51, 59), genomic and plasmid DNA analysis (5, 8, 21, 23, 31, 36–38, 44), and protein analysis (55, 56, 58) have been used to classify pathovars and strains of different species. However, these techniques are often time-consuming, too expensive, or too insensitive for use in routine diagnosis. Therefore, we have been interested in developing new methods to rapidly identify and classify closely related pathogenic bacteria on the basis of genomic fingerprinting approaches.

Families of repetitive DNA sequences are dispersed throughout the genome of diverse bacterial species (32, 40, 62). Three families, unrelated at the DNA sequence level, have been studied in more detail, namely the 35- to 40-bp repetitive extragenic palindromic (REP) sequence (18, 22), the 124- to 127-bp enterobacterial repetitive intergenic consensus (ERIC) sequence (24, 49), and the recently discovered 154-bp BOX element (32, 41). REP, ERIC, and BOX elements have the potential to form stem-loop structures and may play an important role in the organization of the bacterial genome (33, 34, 40). Genome organization is thought to be shaped by selection, and thus the dispersion of the REP, ERIC, and BOX sequences may be indicative of the structure and evolution of the bacterial genome (19, 33, 34, 40). On the basis of this assumption and knowledge about the clonal nature and population dynamics of pathogenic bacteria (1, 8, 16, 37, 38, 48), we hypothesized that if each evolutionary specialized line, or pathovar, of the pathogen had a unique distribution or arrangement of repetitive sequences throughout the genome, we should be able to generate genomic fingerprints that correlate with a specific lineage or pathovar.

In this paper, we demonstrate the utility of the PCR technique with primers corresponding to ubiquitous repetitive DNA sequences (rep-PCR [7, 32, 63]) to generate specific DNA fingerprints of *Xanthomonas* and *Pseudomonas* pathovars and strains. In addition, we show the potential of rep-PCR fingerprinting as a diagnostic tool and in determining whether pathovars represent a single evolutionary line or are composed of several lines that have converged to a similar pathogenic phenotype.

MATERIALS AND METHODS

Bacterial isolates. Sources of and relevant information on bacterial isolates or genomic DNA used in this study are listed in Table 1. All bacteria were stored at −70°C in glycerol and streaked on nutrient-yeast-dextrose agar (*X. campestris* and *X. oryzae* [27]) or King's B agar (*P. syringae* [30]). We focused on pathovars that have been systematically compared and have a similar phenotype, genotype, or host range (3, 4, 21, 35, 52, 53, 59).

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TABLE 1. *Xanthomonas* and *Pseudomonas* isolates or DNA used in this study

Species and pathovar	Isolate[a]	Host	Location	Yr isolated	Source[c]	Reference
X. campestris						
poae	ATCC 33884*[,1]	*Poa trivialis*			ATCC	12
graminis	ATCC 29091*[,1]	*Dactylis glomerata*			ATCC	12
translucens	ATCC 11077*[,1]	Barley			ATCC	
campestris	ATCC 33913*[,1,3]	*Brassica campestris*	UK		ATCC	10
campestris	Xb (B24)[2]	*Brassica* sp.	OR		JH	20
campestris	Xcc 2D520[3]	Wild mustard	CA		JT	20
campestris	JT1	Cabbage	MI	1990	JT	This study
campestris	Xcc 898	Cabbage	MI	1991		This study
phaseoli	ATCC 9563*[,1] (NCPPB 3035)	Bean			ATCC	10
phaseoli	X3S[2] (NCPPB 2064)	Bean			JH	20
phaseoli	Xcp 805	Bean	MI	1992	LA	This study
begoniae	X3[2] (0836i-67, JM)	Begonia	FL	1983	JH	20
pelargonii	X-1	Geranium	KS	1986	KD	9
pelargonii	5-2-4	Geranium	Israel	1987	MD	9
pelargonii	X-5	Geranium	MI	1986	KD	9
pelargonii	5-1-7	Geranium	NY	1987	MD	9
pelargonii	6945-s	Geranium	FL		JBJ	
pelargonii	P6S-390	Geranium	FL	1985	JM	
citri A	XC62[2]	Citrus			JH	20
citri B	XC84[2]	Citrus			JH	20
vesicatoria	Xv31	Pepper	OK	1990	CB	
vesicatoria	Xv91	Pepper	Taiwan		JBJ	
vesicatoria	Xcv 18	Pepper	OH	1992	SM	
vesicatoria	Xv 92-16	Pepper	FL		RS	
vesicatoria	Sp 66	Pepper seed	GA	1993	GO	
vesicatoria	Xv 858	Tomato	MX		JBJ	
vesicatoria	ATCC 11633	Pepper	NJ	1947	ATCC	
vesicatoria	ATCC 35937*[,1]	Tomato	N.Z.	1955	ATCC	10
vesicatoria	ATCC 11551	Tomato	IN	1943	ATCC	
X. oryzae						
oryzae	ATCC 43837**[,1]	Rice			ATCC	52
oryzicola	ATCC 49072*[,1]	Rice			ATCC	52
P. syringae						
morsprunorum	Pm 7	Cherry	MI	1991	AJ	
morsprunorum	Pm 36	Cherry	MI	1991	AJ	
morsprunorum	Pm 567	Cherry	UK		AJ	
syringae	Pss 11	Cherry	MI	1991	AJ	
syringae	Pss 11	Cherry	MI	1991	AJ	
syringae	Pss 66	Cherry	MI	1991	AJ	
syringae	Pss 19	Cherry	MI	1991	AJ	
tomato	Pst 82-14 UGA	Tomato	GA	1982	RG	
tomato	Pst 88-37 UGA	Tomato	GA	1988	RG	
tomato	Pst 88-40 UGA	Tomato	GA	1988	RG	
tomato	Pst 856	Tomato	MI	1992		This study
tomato	Pst 915	Tomato	MI	1993		This study

[a] **, type strain for the species; *, pathovar reference strain; [1], received as DNA from J. Smith, Michigan State University; [2], received as DNA from J. Hartung, U.S. Department of Agriculture, Beltsville, Md.; [3], obtained from J. Tsuji, Michigan State University. Isolate codes in parentheses are known alternative codes.

[b] ATCC, American Type Culture Collection; JH, J. Hartung (see footnote *a*); JT, J. Tsuji (see footnote *a*); LA, L. Afanador, Michigan State University (pathovar isolated from *Phaseolus vulgaris* seed); KD, K. Dunbar, Michigan State University; MD, M. Daughtry, Long Island Horticultural Research Laboratory; JBJ, J. Jones, Gulf Coast Research Center, University of Florida; JM, J. Miller, Florida Department of Agriculture and Consumer Services, Gainesville; CB, C. Bender, Oklahoma State University; SM, S. Miller, Ohio State University; RS, R. Stall, University of Florida; GO, G. O'Keefe, Georgia Department of Agriculture; AJ, A. Jones, Michigan State University; RG, R. Gitaitis, University of Georgia.

Preparation of DNA. Total genomic DNA was prepared by using a modification of the procedure of Ausubel et al. (2). Briefly, cultures were grown in 40 ml of Luria-Bertani medium (46) for 24 to 48 h at 27°C. Cells were lysed in sarcosyl buffer, and the resulting lysate was treated with pronase. DNA was purified with a solution of CTAB-NaCl (10% cetyltrimethylammonium bromide in 1 M NaCl) followed by chloroform and phenol-chloroform extractions and was recovered by isopropanol precipitation, redissolved in TE (10 mM Tris, 1 mM EDTA [pH 8.0]), and quantified spectrophotometrically.

Amplification and separation of DNA bands. Primer sequences corresponding to REP (REP1R-I [5′-IIIICGICGI CATCIGGC-3′] and REP2-I [5′-ICGICTTATCIGGCCTAC-3′]) were provided by J. R. Lupski (63) or synthesized as described below. Primer sequences corresponding to ERIC (ERIC1R [5′-ATGTAAGCTCCTGGGGATTCAC-3′] and ERIC2 [5′-AAGTAAGTGACTGGGGTGAGCG-3′]) were synthesized with a DNA synthesizer (model 380B; Applied Biosystems, Foster City, Calif.) by the Macromolecular Structure, Sequence and Synthesis Facility at Michigan State Uni-

versity. The primer sequence corresponding to BOXA, a subunit of the BOX element (41) (BOXA1R [5′-CTAC GGCAAGGCGACGCTGACG-3′]), was also synthesized at Michigan State University or provided by J. R. Lupski (32). PCR conditions were as previously described (7). The PCR protocols with REP, ERIC, and BOX primers are referred to as REP-PCR, ERIC-PCR, and BOX-PCR, respectively and rep-PCR collectively. PCR amplification was performed with a model 110s Tempcycler II (Coy Corp., Grass Lake, Mich.) by using the following cycles: 1 initial cycle at 95°C for 7 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 44, 52, or 53°C for 1 min with REP, ERIC, and BOX primers, respectively, and extension at 65°C for 8 min with a single final extension cycle at 65°C for 15 min and a final soak at 4°C. PCR mixtures were overlaid with 25 μl of mineral oil (M3516; Sigma).

A 5- to 8-μl portion of amplified PCR product was separated by gel electrophoresis at 4°C on 1.5% agarose gels in 0.75× TAE buffer (46) for 10 h at 5 V/cm, stained with ethidium bromide, and photographed on a UV transilluminator with Polaroid type 55 film. Fingerprints generated from different strains were compared visually.

Reproducibility of DNA fingerprints. Fingerprint profiles generated from independent DNA preparations extracted from single-colony cultures at different times (over a period of several months) were run side by side on an agarose gel to determine their reproducibility. A protocol involving the direct assay of whole cells exuding from plant lesions, suspended in water, or collected directly from solid media was also tested. For this protocol, tomato or geranium leaves with apparent symptoms of bacterial speck or bacterial wilt, respectively, were surface sterilized for 1 min in 0.53% sodium hypochlorite and rinsed three times in sterile distilled water. Lesions were dissected and macerated in 200 μl of water and allowed to stand for 15 min. Then 1 μl of the resultant suspension was added to the PCR mixture. DNA from known pathogenic isolates of *P. syringae* pv. tomato and *X. campestris* pv. pelargonii was used as a positive control and run next to the unknown samples on agarose gels for comparison. For the geranium sample, the resultant suspension was also streaked on nutrient-yeast-dextrose agar and cells from yellow colonies were directly assayed as described below. The REP-PCR profile generated from DNA of an isolate of *X. campestris* pv. vesicatoria (P-93) was compared with the profile generated from the same isolate submitted to our laboratory as a suspension in sterile water. A 1-μl sample of the suspension was added to the PCR mixture. Likewise, samples of cultures suspected to be *X. campestris* pv. pelargonii were submitted as cultures on solid media. Bacterial cells were collected directly from a colony by using a 1-μl disposable inoculating loop and resuspended in the PCR mixture. Fingerprints were resolved on an agarose gel within 24 h of receipt of the sample. DNA of a known *X. campestris* pv. pelargonii isolate was used for comparison.

RESULTS

rep-PCR DNA fingerprints clearly distinguish different pathovars. Primers corresponding to conserved DNA sequences of REP elements, BOXA subunits of BOX elements, and ERIC sequences annealed to genomic DNA and generated unique genomic fingerprints for each pathovar of *X. oryzae* and *X. campestris* tested. The fingerprint patterns of representative strains (Table 1) of *X. oryzae* pv. oryzae and pv. oryzicola and *X. campestris* pv. poae, pv. graminis, pv. translucens, pv. campestris, pv. phaseoli, pv. citri, pv. begoniae, pv. pelargonii, and pv. vesicatoria are shown in Fig. 1 and 2. The REP-, BOX-, and ERIC-PCR yielded 5 to more than 20 distinct PCR products, ranging in size from approximately 100 bp to over 5 kb. Differences among pathovars were assessed visually on the basis of the migration patterns of PCR products.

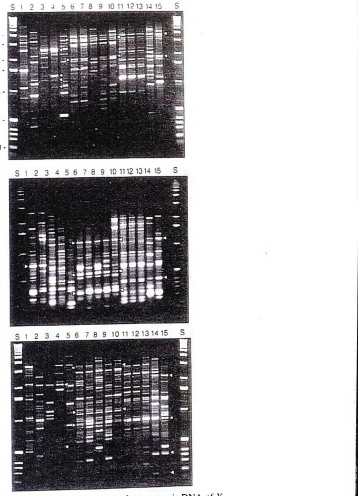

FIG. 1. rep-PCR fingerprinting patterns from genomic DNA of *X. oryzae* and *X. campestris* isolates. The REP-PCR, BOX-PCR, and ERIC-PCR patterns are shown in panels A, B, and C, respectively. A 5- to 8-μl portion of each of the rep-PCR mixtures was loaded onto a 1.5% agarose gel. The resulting electrophoretic patterns of isolates *X. oryzae* pv. oryzae ATCC 43837 (lanes 1) and pv. oryzicola ATCC 49072 (lanes 2) and *X. campestris* pv. poae ATCC 33804 (lanes 3); pv. graminis ATCC 29091 (lanes 4); pv. translucens ATCC 10770 (lanes 5); pv. campestris ATCC 33913 (lanes 6), X6 (lanes 7), 2D520 (lanes 8), JT1 (lanes 9), and 898 (lanes 10); pv. phaseoli ATCC 9563 (lanes 11), X35 (lanes 12), and 805 (lanes 13); and pv. citri A Xc62 (lanes 14) and B Xc 84 (lanes 15) are shown. The control lane, not labeled, represents the same rep-PCR but lacking template DNA. The left and right lanes labeled S show the DNA molecular size marker (1-kb ladder; Gibco-BRL); the sizes are indicated in base pairs. Arrowheads identify similarities or differences among selected isolates as outlined in the text.

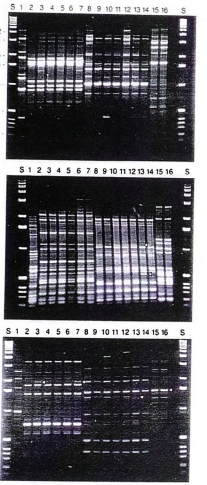

FIG. 2. rep-PCR fingerprinting patterns from genomic DNA of *X. campestris* isolates. The REP-PCR, BOX-PCR, and ERIC-PCR patterns are shown in panels A, B, and C, respectively. A 5- to 8-μl portion of each of the rep-PCR mixtures was loaded onto a 1.5% agarose gel. The resulting electrophoretic patterns of isolates pv. begoniae X3 (lanes 1); pv. pelargonii X-1 (lanes 2), 5-2-4 (lanes 3), X-5 (lanes 4), 5-1-7 (lanes 5), 6945-S (lanes 6), and P85-390 (lanes 7); and pv. vesicatoria isolate Xv31 (lanes 8), Xv91 (lanes 9), Xev18 (lanes 10), Xv 92-16 (lanes 11), Sp 66 (lanes 12), Xv 858 (lanes 13), ATCC 11633 (lanes 14), ATCC 35937 (lanes 15), and ATCC 11551 (lanes 16) are shown. Other details are described in the legend to Fig. 1.

X. oryzae pv. oryzae and pv. oryzicola constitute a single species on the basis of DNA-DNA hybridization studies (52) and share many phenotypic features (60) but incite different symptoms on rice plants (42). REP-, BOX-, and ERIC-PCR clearly differentiated the pathovar reference strains of *X. oryzae* pv. oryzae and pv. oryzicola (Fig. 1, lanes 1 versus lanes 2). Fingerprint profiles generated with each primer set were complex and very different between the two pathovars. In total, over 60 distinct bands were visualized, and only one major PCR product, generated by BOX-PCR and highlighted by an arrowhead (Fig. 1B, lane 1), appeared to comigrate in both strains. Therefore, no obvious relationship between the two pathovars could be surmised on the basis of the REP-, BOX-, or ERIC-PCR fingerprint patterns.

X. campestris pv. poae, pv. graminis, and pv. translucens are closely related on the basis of DNA hybridization and phenotypic studies (53, 59) but are classified as distinct pathovars primarily on the basis of their host range (11, 12, 69). In our study, the pathovar reference strain of *X. campestris* pv. poae yielded distinct fingerprint profiles from those of the reference strain, *X. campestris* pv. graminis (Fig. 1, lanes 3 and 4, respectively), but the presence of several comigrating bands suggested that *X. campestris* pv. poae and pv. graminis are closely related. The REP-PCR profiles of *X. campestris* pv. graminis were comparatively simple, and at least four PCR products, highlighted by arrowheads (Fig. 1A, lane 4), comigrated with bands generated from DNA of *X. campestris* pv. poae. BOX-PCR also yielded multiple bands of parallel mobility (Fig. 1B, lanes 3 and 4). Comigrating bands generated by ERIC-PCR and highlighted by the arrowheads (Fig. 1C, lane 4) were also visible, but the overall patterns were distinct. The REP-, BOX-, and ERIC-PCR profiles of *X. campestris* pv. translucens (Fig. 1, lanes 5) were found to be different from those of both *X. campestris* pv. poae and pv. graminis.

A third example highlighting the ability to detect distinct xanthomonad pathovars is shown in Fig. 2. *X. campestris* pv. begoniae and pv. pelargonii are distinct groups on the basis of serologic (4), host range, phenotypic, protein electrophoretic, and DNA hybridization (58) features. The REP-, BOX-, and ERIC-PCRs (Fig. 2A, B, and C, respectively) generated complex banding patterns from DNA of representative strains of *X. campestris* pv. begoniae (lanes 1) and pv. pelargonii (lanes 2 to 7), and each primer generated very different patterns for the two pathovars.

Pairwise comparisons of strains representative of *P. syringae* pv. morsprunorum, pv. syringae, and pv. tomato demonstrated that the REP-, ERIC-, and BOX-PCR fingerprint profiles were also distinct for different pathovars of *P. syringae*. *P. syringae* pv. morsprunorum and pv. syringae incite the same disease of stone fruits and, when isolated, cannot be differentiated except by dilatory biochemical tests (35) and more recently with a DNA probe (43a). Our fingerprint profiles readily distinguished between the two pathovars (Fig. 3, lanes 1 to 3 versus lanes 4 to 7). Likewise, *P. syringae* pv. syringae, an economically insignificant pathogen of tomato, and *P. syringae* pv. tomato, an economically important pathogen of tomato, can be distinguished by a specific DNA probe (7a) and rare-cutting restriction enzymes (6a) but cannot be rapidly differentiated by other experimental means (26, 27). The REP-, BOX-, and ERIC-PCR fingerprint profiles clearly distinguished strains of *P. syringae* pv. syringae and pv. tomato (Fig. 3, lanes 4 to 7 versus lanes 8 to 12).

Pairwise comparisons of any two pathovars tested demonstrated that strains representative of a particular pathovar yield DNA product patterns that are complex and easily distinguishable from patterns generated from strains belonging to any other pathovar within the species *X. oryzae*, *X. campestris*, and *P. syringae*. Each set of primers (REP, BOX, and ERIC) was effective in distinguishing different pathovars.

Intrapathovar variation. In contrast to the diversity of fingerprints observed among strains representative of different pathovars, each set of primers yielded common banding patterns among isolates within a pathovar. To determine the intrapathovar diversity of DNA fingerprints, we examined several isolates of selected pathovars obtained from geographically distinct locations or isolated from the same geographic area at different times (Table 1).

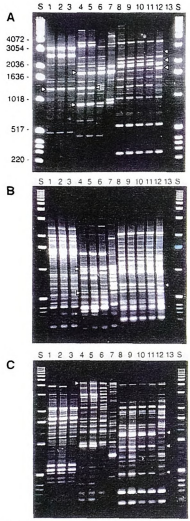

FIG. 3. rep-PCR fingerprinting patterns from genomic DNA of *P. syringae* isolates. The REP-PCR, BOX-PCR, and ERIC-PCR patterns are shown in panels A, B, and C, respectively. A 5- to 8-μl portion of each of the rep-PCR mixtures was loaded onto a 1.5% agarose gel. The resulting electrophoretic patterns of isolates pv. morsprunorum Pm 7 (lanes 1), Pm 36 (lanes 2), and Pm 567 (lanes 3); pv. syringae Pss 11 (lanes 4), Pss 11 (lanes 5), Pss 66 (lanes 6), and Pss 19 (lanes 7); and pv. tomato Pst 82-14 (lanes 8), Pst 88-37 (lanes 9), Pst 88-40 (lanes 10), Pst 856 (lanes 11), and Pst 915 (lanes 12) are shown. Lane 13 shows the control reaction lacking template DNA. Other details are described in the legend to Fig. 1.

The intrapathovar diversity of the isolates tested could be grouped into two broad categories: (i) pathovars from which isolates had nearly identical REP, BOX, and ERIC fingerprints or from which isolates had overall unique profiles but shared multiple bands of apparent equal mobility, and (ii) pathovars from which isolates could be divided into groups (i.e., evolutionary lines) that did not share common REP-, BOX-, or ERIC-PCR banding patterns.

Most of the pathovars were found to belong to the first category. For example, no notable differences were observed between the three isolates of *X. campestris* pv. phaseoli (Fig. 1, lanes 11 to 13) or between the three isolates of *P. syringae* pv. morsprunorum tested (Fig. 3, lanes 1 to 3). The analysis of six isolates of *X. campestris* pv. pelargonii by the BOX-PCR yielded identical profiles (Fig. 2B, lanes 2 to 7). The REP-PCR yielded a single additional band for isolates 5-2-4, 5-1-7, and P85-390 compared with the other three isolates tested (Fig. 2A, lanes 2 to 7). The ERIC-PCR of isolates 5-2-4 (Fig. 2C, lane 3) and P85-390 (Fig. 2C, lane 7) yielded a single polymorphism (arrowheads) in contrast to the other isolates. For the five isolates of *P. syringae* pv. tomato tested (Fig. 3, lanes 8 to 12), two patterns were apparent. Isolate 915 demonstrated some polymorphisms (accentuated by arrowheads in Fig. 3, lanes 12) in contrast to the other four isolates examined, but the majority of bands were analogous. Two distinct groups of *P. syringae* pv. tomato strains have also been identified by restriction fragment length polymorphism analysis (8) and field inversion gel electrophoresis of DNA digested with rare-cutting enzymes (6a).

Each *X. campestris* pv. campestris and pv. citri isolate tested had unique REP-, BOX-, and ERIC-PCR fingerprint profiles, but the presence of multiple bands of apparent equal mobility suggested that isolates within each pathovar had a common evolutionary heritage. For example, the five isolates of *X. campestris* pv. campestris (Fig. 1, lanes 6 to 10), including the type strain for the pathovar and species (ATCC 33913 [lanes 6]), shared multiple comigrating bands (accentuated by opposing arrows in lanes 6 and 10) but were highly diverse for the remainder of the bands generated. Isolate X6, (lanes 7) was highly similar to JT1 (lanes 9), and both were similar to the ATCC type strain (lanes 6). Likewise, the *X. campestris* pv. citri pathotype A isolate (Xc62; lanes 14) and the pathotype B isolate (Xc84; lanes 15) shared PCR products of equal mobility (accentuated by arrowheads in lanes 15), but the overall patterns were quite distinct (compare lanes 14 and 15).

The four isolates of *P. syringae* pv. syringae (Fig. 3, lanes 4 to 7) had a number of REP-, BOX-, and ERIC-PCR products in common, accentuated by the arrowheads in lanes 4, but multiple additional bands were generated to yield strain-specific profiles. Isolate Pss 11 (lanes 4) and Pss 11 (lanes 5) yielded apparently identical profiles with each type of primer set. Isolate Pss 66 (lanes 6) was highly similar to Pss 11 and Pss 11 but yielded a number of unique PCR products. The BOX-PCR (Fig. 3B) profiles appeared more similar among these three isolates than did the REP-PCR (Fig. 3A) and ERIC-PCR (Fig. 3C) profiles. In contrast, the REP-, BOX-, and ERIC-PCR patterns generated from DNA of Pss 19 (lanes 7) were very different from each of the other three isolates, although common bands (accentuated by the arrowheads in lanes 4) of equal mobility could be identified.

The REP-, BOX-, and ERIC-PCR protocols provided similar conclusions about the apparent relatedness among isolates that yielded similar fingerprint profiles. When isolates within a pathovar demonstrated polymorphisms, each primer set offered unique information, generating strain-specific profiles.

The second category of diversity was observed within the pathovar *X. campestris* pv. vesicatoria. The REP-, BOX-, and ERIC-PCR protocols demonstrated that this pathovar is composed of at least two distinct groups (Fig. 2, lanes 8 to 16). Within the first group (group A [lanes 8 to 14]), isolates obtained over time and from distant geographic sources including Taiwan (Xv 91 [lanes 9]), Mexico (Xv 858 [lanes 14]), and several states of the United States including Oklahoma (Xv 29 [lanes 8]), Ohio (Xcv 18 [lanes 10]), Florida (Xv 92-16

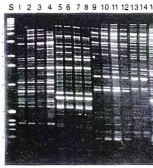

FIG. 4. ERIC-PCR fingerprint patterns of *X. campestris* pv. campestris and pv. pelargonii and *P. syringae* pv. syringae isolates generated from different DNA preparations from single-colony cultures of the same strain. The resulting electrophoretic patterns of isolates *X. campestris* pv. campestris JT1 (lanes 1 and 2) and 388 (lanes 3 and 4); *X. campestris* pv. pelargonii X-1 (lanes 5 and 6) and 5-2-4 (lanes 7 and 8); and *P. syringae* pv. syringae Pss 11 (lanes 9 and 10), Pss 11 (lanes 11 and 12), Pss 66 (lanes 13 and 14), and Pss 19 (lanes 15 and 16) are shown. Other details are described in the legend to Fig. 1.

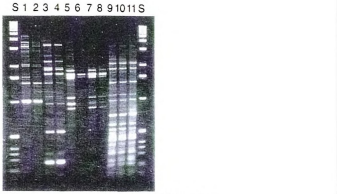

FIG. 5. Comparison of whole-cell rep-PCR with rep-PCR in which purified genomic DNA was used. A 3- to 10-μl portion of each of the rep-PCR mixtures was loaded onto a 1.5% agarose gel. The resulting REP-PCR electrophoretic patterns generated from *X. campestris* pv. vesicatoria P-93 DNA (lane 1) or 1 μl of a cell suspension in water (lane 2); *P. syringae* pv. tomato 915 DNA (lane 3) or 1 μl of a cell suspension exudate from a lesion on a tomato leaf (lane 4); *X. campestris* pv. pelargonii 803 DNA (lane 5), 1 μl of a cell suspension exudate from a lesion on a geranium leaf (lane 6), or cells collected directly from solid media (lanes 7 and 8); and BOX-PCR products generated from *X. campestris* pv. pelargonii 803 DNA (lane 9) or from cells collected directly from solid media (lanes 10 and 11) are shown. Other details are described in the legend to Fig. 1.

[lanes 11]), Georgia (Sp 66 [lanes 12]), and an ATCC strain (ATCC 11633 [lanes 14]) were very similar to each other, with very few unique bands. No major differences were noted among these strains when the REP-PCR protocol was used (Fig. 2A), but the presence or absence of one or more unique bands was noted when the BOX-PCR (Fig. 2B) and ERIC-PCR (Fig. 2C) protocols were used, as accentuated by the arrowheads in Fig. 2B and C, lanes 13.

The second group (group B), including the reference strain for the pathovar (ATCC 35937), originally isolated in New Zealand by D. W. Dye in 1955 (Fig. 2, lanes 15), and strain ATCC 11551, isolated in 1943 in Indiana (lanes 16), appeared to have identical fingerprint patterns but were very distinct from isolates in group A. Isolates in groups A and B appeared to share only one single band of equal mobility, as generated by the ERIC-PCR and highlighted with an arrowhead in Fig. 2C, lanes 16. Although it cannot be ruled out that this comigration pattern is fortuitous in this particular case, such disparate fingerprint profiles may indicate cases in which two or more evolutionary lines have converged to a similar pathogenic phenotype and have been erroneously classified within the same taxon.

Reproducibility of DNA fingerprints. DNA fingerprints for specific strains could be consistently generated from a single preparation of DNA (data not shown). To determine the reproducibility of generating fingerprint profiles from different DNA preparations of the same strain, we analyzed DNA from cultures initiated from single colonies and extracted at different times (over a period of several months). An example of this analysis, using ERIC-PCR, is shown in Fig. 4. Strains that yielded some of the most complex fingerprint patterns were used for this purpose, and in each case identical profiles were generated (compare lanes 1 and 2, lanes 3 and 4, etc.). The single ERIC-PCR polymorphism noted among isolates of *X. campestris* pv. pelargonii (Fig. 2C) had been of particular interest. This polymorphism was reproducible (see the arrowhead in Fig. 4, lanes 5 and 6 versus lanes 7 and 8).

To determine if whole cells, as opposed to purified DNA, could be used for rep-PCR, we compared the rep-PCR patterns resulting from the two protocols. The quality of DNA preparations did not affect fingerprint profiles substantially, since whole cells yielded similar profiles to those generated from isolated DNA (Fig. 5). Analogous REP-PCR and BOX-PCR genomic fingerprints were generated from purified genomic DNA and from cells suspended in water, derived from cultures on solid media, or exuded from plant lesions. Figure 5 shows the REP-PCR profile generated from DNA of *X. campestris* pv. vesicatoria (lane 1) isolate (P-93) compared with the profile generated from cells suspended in water (lane 2). Lanes 3 and 4 show the fingerprint generated from DNA of *P. syringae* pv. syringae 915 (lane 3) compared with the profile generated from cells collected from a plant lesion (lane 4). Likewise, lanes 5 through 8 show the REP-PCR pattern generated from DNA of *X. campestris* pv. pelargonii 803 (lane 5) compared with the profile generated from cells collected from a plant lesion (lane 6) or from cells collected from solid media (lanes 7 and 8). Positive diagnosis was achieved within 24 h if a profile could be generated directly from infected plant tissue or after 2 to 4 days if bacteria were first cultured on a solid medium. Several tomato and geranium plants collected from commercial greenhouses or submitted to the Michigan State University plant-diagnostic laboratory were evaluated in this manner to rapidly identify the causal organism. Finally, two bacterial cultures on solid media and thought to be *X. campestris* pv. pelargonii yielded BOX-PCR profiles (Fig. 5, lanes 10 and 11) identical to those of a known *X. campestris* pv. pelargonii isolate (803 [lane 9]). Positive confirmation of the identity of the isolates was achieved within 24 h of sample submission.

DISCUSSION

In this study we have demonstrated that repetitive extragenic sequences such as REP, ERIC, and BOX are present in the genome of diverse *Xanthomonas* and *Pseudomonas* strains.

confirming and extending the conclusion of Versalovic et al. (63), de Bruijn (7), and Koeuth et al. (32) that these sequences are virtually ubiquitous. We have also demonstrated that the REP-, ERIC-, and BOX-PCR protocols, referred to as rep-PCR collectively, are particularly suitable for the rapid molecular characterization of plant-pathogenic bacteria, especially at the pathovar level. The rep-PCR protocol clearly has the potential to differentiate pathovars, including those that are not easily distinguished by other phenotypic and phylogenetic techniques. rep-PCR may also be capable of discerning whether pathovars represent a single evolutionary line or are composed of several lines that have converged to exhibit a similar pathogenic phenotype.

The data presented here suggest that rep-PCR should also be a useful tool for diagnostic purposes in plant pathology. Similar suggestions have been made about the utility of rep-PCR in infectious-disease diagnosis and epidemiological analysis in human pathology (62, 64, 67). Several criteria must, of course, be met if rep-PCR is to be useful for the proper identification of unknown isolates. First, the characteristic location of REP-, ERIC-, and BOX-like sequences, and therefore the genomic fingerprint patterns generated by rep-PCR, must be stable over time and distance. Our results suggest that this is the case. For example, the ATCC 11633 isolate of *X. campestris* pv. vesicatoria group A was first isolated in the late 1940s, and isolates obtained from Taiwan, Mexico, and various locations in the United States over the last several years (Table 1) have nearly identical fingerprint profiles (Fig. 2). Likewise, we have analyzed isolates of *X. campestris* pv. vesicatoria collected in 1993 during epidemics in Michigan that have a similar profile to the *X. campestris* pv. vesicatoria group B isolates (Fig. 2), which date back to 1943 and 1955 (data not shown). Comparison of the genomic fingerprint profiles of other isolates within a pathovar, separated by time or distance (Table 1), supports the notion that the profiles remain stable. The similarity of fingerprint profiles from related isolates separated by time has been noted by others (67).

Second, the rep-PCR technique must be able to discriminate among related but distinct bacterial strains with sufficient resolution and be reproducible. We have demonstrated that closely related bacteria, as determined by other experimental protocols such as DNA hybridization studies, can have divergent rep-PCR profiles and that bacteria associated with the same host or disease complex can be easily differentiated because of the complexity of DNA amplification products generated by using the three different primer sets. Identical fingerprint profiles generated from DNA isolated from single-colony cultures of an isolate (Fig. 4) and from isolates separated by time or distance (e.g., *X. campestris* pv. pelargonii [Fig. 2]) support the reproducibility of the rep-PCR protocol.

Third, background work with large numbers of isolates will have to be done to ascertain the homogeneity or heterogeneity of fingerprint profiles within each pathovar.

We systematically compared the general utility of REP, BOX, and ERIC primers (Fig. 1 to 3). In general, differences between pathovars and substantial polymorphism (dissimilarities between strains within a pathovar) were detected by each primer set with similar resolution, suggesting that the distribution of REP, ERIC, and BOX sequences is a true reflection of genomic structure. For detecting limited polymorphisms within a clonal group or apparent similarities between pathovars, each primer set offered unique information. For example, REP-PCR suggested that *X. campestris* pv. poae and pv. graminis were closely related, whereas this was less obvious with ERIC-PCR. The REP- and ERIC-PCR fingerprints of isolates of *X. campestris* pv. campestris were highly diverse, but analogous profiles were more obvious when BOX-PCR was used. By using three different primer sets, a broader survey of the chromosomal structure was possible and more specific conclusions concerning diversity or similarity among strains and pathovars were achieved.

Reproducible fingerprint profiles of a particular strain can be generated from DNA isolated at different times from the same colony, from different colonies of the same strain, or from serial cultures of the same strain (47; also see above). However, the rep-PCR protocol is subject to the same experimental caveats as all other PCR-based techniques, such as the problem of amplifying even minute amounts of contaminating DNA, variations in template and primer DNA concentrations, and the composition of the PCR buffer. Moreover, the electrophoresis conditions can influence the interpretation of results. Therefore, we recommend that the rep-PCR be carried out at least two independent times with appropriate negative controls (e.g., no template DNA in the reaction mixture and/or no primer DNA) and that the rep-PCR products be separated on at least two independent gels (47).

In this study, a limited amount of variation in rep-PCR patterns, probably as a result of the above limitations, was observed. For example, more faint bands generated from DNA of the *P. syringae* pv. syringae strains with ERIC-PCR were observed in Fig. 5 (lanes 9 to 16) than in Fig. 3C (lanes 4 to 7). In general, depending on the efficiency of the PCR, large bands (greater than 5 to 6 kb) and faint bands were not always amplified to the same extent or were not equally visible after ethidium bromide staining. More prominent bands, however, were consistently present and were almost always sufficient to identify specific pathovars or strains and able to distinguish them from closely related strains.

Other PCR-based genomic fingerprinting techniques have been described recently. For example, arbitrary primers and PCR (65, 66), also known as random amplified polymorphic DNA (RAPD) analysis, has a demonstrated use for differentiating bacteria and for diagnostic purposes (6, 13). There are some crucial differences between rep-PCR and RAPD-type analyses that are particularly relevant when considering the reproducibility of diagnostic protocols. The major difference lies in the length of the primers used and the corresponding PCR conditions. RAPD analysis relies on the use of primers with arbitrary sequences (most commonly 10-mers but up to 34 bp [65, 66]), whereas rep-PCR involves the use of primers of 18 to 22 bp with high homology to repetitive sequences (32, 63). The latter primers allow the use of more stringent PCR conditions, which, in turn may reduce experimental variation and PCR artifacts.

In this study, we also show that it is not necessary to use purified genomic DNA as a template for rep-PCR. Bacterial cells derived from single colonies on agar plates or from liquid cultures or eluted directly from pathogen-induced lesions on plants could be used directly for rep-PCR. Apparently, the cycling of the reaction at 95°C released enough DNA from the cells to serve as a template for rep-PCR. In fact, rep-PCR with bacterial cells yielded genomic fingerprinting patterns indistinguishable from those generated with purified template DNA. This phenomenon has been observed with a variety of gram-negative and gram-positive bacteria (47, 68) and with plant (nodule) tissues infected with symbiotic *Rhizobium*, *Azorhizobium*, or *Bradyrhizobium* isolates (45, 47). This form of "whole-cell rep-PCR" (68) is particularly useful for rapid and routine diagnostic analyses.

Reproducible and routine rep-PCR-based genomic fingerprinting for the identification of phytopathogenic bacteria is expected to be further facilitated by the use of fluorescently

labeled REP, ERIC, and BOX primers and the Applied Biosystems DNA Sequenator equipped with the Genescan software package, as has been demonstrated for human DNA analysis using fluorescently tagged short tandem repeats (14). Preliminary results suggest that the generation of "bar code-like" genomic fingerprints of bacteria and their storage in data files by this method is possible (39, 61).

Although the rep-PCR technique is very useful for bacterial strain identification, the utility of REP-, BOX-, and ERIC-PCR for bacterial taxonomy may be limited to closely related strains, as noted by others (7, 28, 67). For assessing the genetic distance between diverse bacteria, rRNA gene-based techniques are likely to be more useful (5, 25). Conclusions about evolutionary relatedness by rep-PCR, especially on the basis of a few comigrating bands, would have to be confirmed by DNA sequencing or hybridization analysis. In this study, few common bands among pathovars, even those known to be closely related, were observed. Of particular interest were the two pathovars of *X. oryzae* (Fig. 1, lanes 1 and 2). *X. oryzae* pv. oryzae and pv. oryzicola have a DNA hybridization value greater than 90% (52) and cannot be reliably distinguished in 129 of 133 phenotypic tests (60). However, each pathovar has specialized to incite unique symptoms on rice plants (42). This specialization may be correlated with the distribution of REP, BOX, and ERIC sequences to the extent that a conclusion of common ancestry cannot be made on the basis of the resultant fingerprint profiles. Approaches other than pathogenicity tests, such as protein profile analysis or fatty acid profile analysis (59), serologic testing (3), and rRNA gene restriction patterns (5), support the distinctiveness of *X. oryzae* pv. oryzae and pv. oryzicola.

X. campestris pv. poae and pv. graminis (Fig. 1, lanes 3 and 4) offer another unique comparison. These pathovars, including the strains used in our study, have similar fatty acid profiles (59) and cannot be distinguished in 215 of 257 phenotypic tests (53). However, the pathovars have distinctive sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of whole-cell proteins and are accepted as distinct pathovars. The primary criterion for splitting the pathovars is based on host range studies (12). The rep-PCR fingerprint profiles between the two pathovars contain many bands of equal mobility, but they are distinct, consistent with the concept that selection for a specialized niche affects genome organization (33) and that this corresponds to a unique distribution of repetitive sequences in the bacterial genome.

The rep-PCR technique may be limited for phylogenetic analysis, but it effectively differentiated two evolutionary lines classified within the same taxon of *X. campestris* pv. vesicatoria. Two distinct groups have also been noted by Vauterin et al. (57) and Stall et al. (50).

In conclusion, we have found that REP-, BOX-, and ERIC-like sequences are prevalent in strains of *X. oryzae*, *X. campestris*, and *P. syringae* and can be exploited to generate genomic fingerprints of strains within each species. Selection for a specialized niche appears to affect the distribution of repetitive sequences, resulting in fingerprints unique to specific pathovars or strains. Unique fingerprint profiles generated by rep-PCR can be exploited for diagnostic purposes and for discerning evolutionary lines that make up a pathovar. Disclosing the population diversity of each pathovar, in turn, has implications for the implementation of breeding programs, other disease management strategies, and ecological and epidemiological studies.

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