Reducing copper and chlorothalonil in staked tomato production on Virginia's Eastern Shore

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By

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(Abstract)

Virginia ranks third in fresh market staked tomato (*Lycopersicon esculentum* Mill.) production with approximately 1,659 hectares on the Eastern Shore. Estimated annual gross value is $30,800,000. Copper and chlorothalonil have long been considered essential to control bacterial and fungal diseases in fresh market tomatoes. High rates of these fungicides on tomatoes grown under plastic mulch have led to concerns about their potential adverse effect on water quality in estuaries adjacent to fields. The development of new fungicides, such as azoxystrobin and acibenzolar-S-methyl, which have more favorable environmental fate characteristics and are used at much lower rates, may provide viable alternatives to copper and chlorothalonil. Using a disease forecasting system, such as *Tomcast*, may reduce the number of applications of fungicides.

The research objectives of this study were to reduce the amount of copper and chlorothalonil used in fresh market tomato production and to evaluate the effectiveness of the *Tomcast* disease forecasting system for controlling fungal leaf diseases on the Eastern Shore. Field studies compared copper to acibenzolar-S- methyl for bacterial diseases caused by *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*. Research plots were established in a randomized complete block design with four

replications in grower fields and at the Eastern Shore Agricultural Research & Extension Center. Acibenzolar proved to be as effective as the standard copper bactericides in controlling bacterial spot. Acibenzolar provided better control than the standard copper bactericides when bacterial speck was the target disease. Azoxystrobin application alternated with maneb was evaluated as a replacement for chlorothalonil. Azoxystrobin and *Tomcast* were studied as tools to reduce chlorothalonil use for control of *Alternaria solani*. *Tomcast* can reduce the number of applications by 40-70 % per year and provide adequate control of early blight. Azoxystrobin provides better control of early blight than chlorothalonil. Use of these new, more environmentally compatible, plant-protection products , along with the *Tomcast* disease forecasting system, can significantly reduce or eliminate the need to use copper and chlorothalonil for tomato disease management and therefore eliminate them as potential pollutants of the Chesapeake Bay and Atlantic Ocean estuaries.

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I. Literature Review

I-1.1 Introduction

The Eastern Shore of Virginia consists of two counties: Accomack and Northampton. These counties are situated at the southern tip of the Delmarva Peninsula. Virginia's portion of the peninsula is 120 km long and averages approximately 11 km in width. Elevation ranges from sea level to 14.6 meters. The 50-year average for rainfall is 121 cm per year. The Chesapeake Bay is to the west and the Atlantic Ocean to the south and east of the peninsula. There are 780 km of shoreline on the Eastern Shore with 468 km of shoreline in Accomack County alone (Anonymous, 1982). Extensive estuary systems are located throughout the Eastern Shore landmass. The estuaries are used to produce clams and oysters and to harvest natural inhabitants for seafood. There are 261 saltwater farms in Virginia, comprising 3,106 ha (Anonymous, 1998b).

The soil of the Eastern Shore is primarily Bojac sandy loam with less than 1% organic matter. The soil is very well suited for growing row crops and vegetable crops, but is subject to run-off and leaching. Run-off from agricultural fields has been suspected to be the cause of high clam larvae mortality in Accomack County. Preliminary data indicates that run-off from selected plasticulture fields and the water in adjacent creeks contain toxic concentrations of copper and chlorothalonil (Dietrich et al., 1996) and that creek water near selected fields is toxic to sentinel species, such as grass shrimps (Luckenbach et al., 1996). Concentrations of copper that are toxic to shellfish larvae when added to seawater ranged from 16 to 33 ug/L (Dietrich et al., 1996). The toxicity of copper and chlorothalonil to *Daphnia magna*, bluegill, and mammals is described in Table I-1.

I-1.2 Staked Tomato Culture on the Eastern Shore of Virginia

The Eastern Shore is dominated by crop production, which includes corn, soybeans, cotton and small grains, along with a prosperous vegetable industry. The vegetable industry on the Eastern Shore is dominated by 1,659 ha of staked tomatoes worth $30.8 million (Manheimer and Vick, 2001). This comprises 99% of Virginia's tomato production. Virginia ranks third in fresh market tomato production behind California and Florida (Manheimer and Vick, 2001). Other vegetables produced in abundance are potatoes at 2,630 ha and snap beans at 2,144 ha. Smaller amounts of peppers (364 ha), watermelons (728 ha), squash, sweet potatoes (202 ha), spinach, and cucumbers (1,618 ha) can be found (Manheimer and Vick, 2001).

Tomatoes grown on the Eastern Shore are produced in a plasticulture system as are many crops worldwide. Plasticulture, first reported in Florida in 1966, is the practice of using polyethylene mulch to cover soil and/or the crop being grown (Anonymous, 1966). Virginia growers have been using plasticulture since the early 1970s, and this production system is being used there annually on several thousand hectares of tomatoes. Plasticulture has become universally popular for growing certain tomato varieties for vine-ripe and fresh markets. In addition to tomatoes, which represent the highest valued crop of the Eastern Shore, peppers, eggplant, watermelons, squash, pumpkins, and cucumbers are also grown under plastic mulch.

I-2 Important Bacterial Pathogens

I-2.1 Bacterial Speck

Pseudomonas syringae pv. *tomato* (*P.s.* pv. *tomato*) causes the disease, bacterial speck of tomato (*Lycopersicon esculentum*, Mill). The pathogen was described in 1933 in the U.S. and Taiwan. It was described in Taiwan as *Bacterium tomato* (Okabe, 1933) and in the U.S. as *Bacterium punctulans* (Bryan, 1933). It was transferred to the genus *Pseudomonas* in 1948 by Burkholder (Reid, 1948). *Pseudomonas* means "false unit" and *syringa*e is derived from the genus name for lilacs (*Syringa*) (Bergey, 1984). The pathovar name *tomato* is derived from the name of the host plant the bacterium infects.

I-2.2 Symptoms of Bacterial Speck

Lesions on green fruit consist of slightly raised dark specks. Only the young, green fruits are susceptible (Walker, 1952). The epidermis is raised but not broken, and as the fruit approaches the pink stage, the tissue around the spot retains its green color longer than normal. Small, black spots appear on leaves, stems, petioles, pedicels, and peduncles. On the peduncle, specks tend to coalesce to form large spots.

I-2.3 Disease Cycle

P.s. pv. *tomato* can survive in the soil, on plants, and in aerosols, as well as inside host plants. *P.s.* pv. *tomato* has been classified as both a soil inhabitant and a soil invader (Bashan et al., 1978; Bosshard-Heer and Vogelsanger, 1977; Schneider and Grogan, 1977.) *P.s.* pv. *tomato* survives preferentially in native weeds and can be isolated from the rhizospheres of non-host crop plants (Devash et al., 1980; Schneider and Grogan,

1977; Valleau et al., 1944). Species of jimsonweed and nightshade on the Eastern Shore may be hosts of *P.s.* pv. *tomato.*

Under hot, dry weather conditions, the bacterial speck pathogen survives as a leaf resident. Trichomes provide important long-term survival and multiplication sites for the pathogen on dead plant tissue (Bashan et al. 1981) and serve as a major habitat for survival of *P.s.* pv. *tomato* during dry conditions (Schneider and Grogan, 1977). When temperature and moisture are favorable, the resident population multiplies and infection can take place (Schneider and Grogan, 1977). McInnes et al. (1988) showed that clipping and harvesting increased levels of airborne bacteria and aided in the dispersal of epiphytic populations. Chemical-control applications may also promote production of aerosols.

Fruit infection occurred, starting at the open corolla stage, when plants were inoculated in field and greenhouse studies (Getz et al., 1983b). Fruit inoculated when pink or red did not develop lesions. Susceptibility of the fruit decreased as size increased. Getz et al. (1983b) found that fruit are most susceptible after anther formation up to a size of 3 cm in diameter. In earlier research, Getz et al. (1983a) found that non-injured fruit are infected through openings that remain after trichomes are shed and before the cuticle is fully developed.

I-2.4 Bacterial Spot

The pathogen, *Xanthomonas campestris* pv. *vesicatoria* (*X. c.* pv. *vesicatoria*), causes the disease, bacterial spot of tomato. The genus name *Xanthomonas* is derived from the Latin terms *xanthus,* which means yellow, and *monas,* which means unit

(Bergey, 1984). The species epithet v*esicatoria* means scab-forming or vesicle-producing (Dowson, 1957). The pathogen has gone through the following name changes: *Bacterium vesicatorium* (1920), *Pseudomonas vesicatoria* (1925), *Phytomonas vesicatoria* (1935), *Bacterium exitiosum* (1921), *Pseudomonas exitosa* (1921), and *Phytomonas exitosa* (1923) (Elliott, 1951).

Currently, there is controversy regarding the naming of this pathogen. Schaad et al. (2000) propose that *X. vesicatoria* type A be retained *as X. campestris* pv. *vesicatoria* and *X. vesicatoria* type B be named *X. exitosa*. However, Jones et al. (1998) suggest that the name *Xanthomonas axonopodis* pv. *vesicatoria* be used to describe races T1 and T3, while the name *Xanthomonas vesicatoria* be used to describe race T2. The races are named for the crop and the avirulence gene the pathogen over comes while types A-D were named for their host range. Most recently, Vauterin et al. (2000) showed evidence that supports division of *X. c*. pv. *vesicatoria* into two species, *X. axonopodis* and *X. vesicatoria*. In the first chapter of this research, the names of the pathogen will be used as they are stated in the literature referenced. However, in the Results and Discussion Chapters, the pathogen will be named as described by Jones et al. (1998), *X. c*. pv. *vesicatoria*, referring to all pathogens that cause bacterial spot.

I-2.5 Symptoms of Bacterial Spot

Lesions on the leaves are at first small, water-soaked dots, which become irregularly circular with yellowish, translucent borders. The centers become brown to black, sunken, and later parchment-like. Spots, when numerous, may coalesce and form irregular, discolored streaks along the veins or margins. Edges and tips of leaves may

become dead and dry, break away, and give the leaves a ragged appearance. Heavily colonized leaves turn yellow or brown and young leaves become distorted and die.

Fruit lesions may occur at any point on the surface. They first appear as small brown or black, raised dots or blisters with narrow, water-soaked margins. They enlarge from 1 to 3 mm, and form slightly elevated, blackened, superficial scabs with irregularly lobed margins and water-soaked halos. The central tissue becomes brown, corky and scab-like and may become so sunken that the lesions resemble pits or craters. A sticky exudate may ooze from the lesion. Blotch-like, scabby areas may result where smaller spots coalesce, but there is no marked malformation or cracking of fruit.

Lesions may occur on many parts of the plants. On cotyledons, lesions are small, sunken, silvery spots, which later turn darker in color. Lesions on stems, petioles and the rachis are elongated and blackened, and may cause death of leaflets. Cankers on older parts of the stem are at first irregular, dark green, water-soaked areas, which become corky, slightly raised, roughened, and cracked longitudinally (Elliott, 1951).

I-2.6 Source of Inoculum of *Xanthomonas campestris* pv. *vesicatoria*

The pathogen survives between seasons within lesions on volunteer plants, weeds, and seeds. Solanaceous plants and other weed hosts harbor the bacteria either as epiphytes or in restricted lesions. The bacterium does not compete well with soil bacteria and disappears from soils as the vegetative material containing the bacterium decomposes (Jones et al., 1986). The bacteria travel from plant to plant in wind-blown water. They are dispersed locally by thundershowers and long-distance by hurricanes (Swings and

Civerolo 1993). *X. c.* pv. *vesicatoria* has also been shown to overwinter on the roots of wheat (Diachun and Valleau, 1946).

I-2.7 Disease cycle of Bacterial Spot

Bacterial spot of tomato occurs wherever tomatoes and peppers are grown. The disease is severe in climates with high rainfall and high temperature (Bergey 1984).

Little is known about epiphytic sites of the bacteria. Bacterial cells have been observed in depressions between epidermal cells and on the basal parts of leaf hairs, but rarely near the guard cells or in stomatal openings (Hirano and Upper, 1983). Bacterial cells of *X. c*. pv. *vesicatoria* on pepper are bound to the fruit surface by fibrillar material (Bashan and Okon 1986). Romantschuk (1993) reported that *X c*. pv. *vesicatoria* produced fimbriae or pili that attached to plant surfaces. These attachment mechanisms appear to increase and stabilize the resident population. Recently it was shown that epiphytic populations of *X. c*. pv. *vesicatoria* can survive better on buds, where copper does not reach and where colonies are protected from harmful ultraviolet radiation (Pernezy and Collins, 1997).

Xanthomonads lack an active mechanism for penetrating the protective barriers of plants. Therefore, the pathogen can only enter through wounds and natural openings, which are primarily stomata and hydathodes. The mechanisms used for penetration are chemotaxis and aerotaxis, rapid multiplication in the substomatal cavity, or a passive ingress with the guttation fluid.

The amount of time a stomate is open and the numbers of stomata on a leaf are directly related to the number of lesions on an inoculated leaf (Ramos and Volin, 1987).

It was found that when stomata were chemically closed, fewer lesions formed after inoculation (Ramos and Volin, 1987). In the same study, plants with fewer stomata produced fewer lesions.

Wounds from stringing, thinning, insects, wind-blown sand, broken leaf hairs, and picking provide avenues of ingress for the pathogen (Wallis et al., 1973). The pathogen spreads more rapidly when plants are wet (Prohozeny et al., 1990). Immature fruit and leaves are more susceptible than mature tissues (Nayudu and Walker, 1960). Wounds from high-pressure sprays can also increase the ingress of the pathogen (Vakili, 1967).

The optimal temperature for growth of the pathogen is 30^0 C. The maximum temperature is 40^0 C and the minimum temperature is 5^0 C for growth (Elliott, 1951). These temperatures are *in vitro* observed temperatures, and *in viv*o temperatures may vary. Maximum bacterial populations have been estimated at $1x10^8$ to $3x10^9$ bacterial cells per cm^2 of leaf.

Moisture from rainfall, irrigation, dew, fog, and plant-water congestion at the hydathode is necessary for bacterial survival, dispersion, invasion of host tissue, and growth within the plants. When raindrops hit a lesion, bacteria are easily suspended in water, increasing chances for bacteria survival and infection efficiency (Wiles and Walker, 1952). Populations of *X. c.* pv. *vesicatoria* increased 10 – 100-fold on tomato leaves at high relative humidity (RH) (>90%). They fluctuated erratically at moderate RH (50-65%) and declined to very low levels at low RH (10-25%) (Timmer, 1989).

I-2.8 Life Cycle of *Xanthomonas campestris* pv. *vesicatoria*

The life cycle of *X. c.* pv. *vesicatoria* consists of three main phases: the epiphytic phase, the pathogenic phase, and the saprophytic phase. During the epiphytic phase,

bacteria reside on the plant surface. Disease expression occurs during the pathogenic phase and the saprophytic phase occurs in the absence of the host.

During the epiphytic phase the pathogen multiplies just enough to survive until conditions become favorable for infection.

The pathogenic phase of *X. c.* pv. *vesicatoria* is the most important phase of the pathogen's life cycle. This phase consists of the endophytic population growth stage, which includes bacterial penetration and movement inside the plants, along with exponential multiplication. Lesions form during this phase.

The saprophytic phase of the pathogen has been recognized as a form of winter survival. In this phase, populations of the bacteria become static or decline (Stall and Cook, 1966). During the survival phase, the bacterium produces an exopolysaccharide (EPS) slime, which behaves as a hydrophilic colloid. The water-holding capacity of the EPS may help the bacterium survive during unfavorable conditions. During this survival phase, the bacteria undergo a state of reduced metabolism that is termed hypobiotic, a state in which nutrients are not needed (Swings and Civerolo, 1993).

I-2.9 Copper Resistance

Resistance to copper, zinc and antibiotics has been an important issue in controlling bacterial pathogens since the use of antibiotics began in the 1950s. Resistance is transferred throughout bacterial populations by plasmids. Lai et al. (1977) obtained transmission of plasmids, RP4 and RK2, from *Escherichia coli* to *X. campestris* pv. *vesicatoria*. These plasmids conferred resistance to various antibiotics and production of penicillinase. The trans-conjugants were able to transmit the plasmids to

other strains of the same pathovar to other pathovars and to bacterial phytopathogens belonging to other Gram-negative genera.

In 1999, it was found that genes for copper resistance were located on the chromosome of strain XvP26 of *Xanthomonas axonopodis* pv. *vesicatoria* (Basim et al., 1999). More importantly, this gene may be transferred between cells through conjugation. This type of conjugation could explain the diversity among strains of the bacterial spot pathogen. Sudden shifts in races from year to year have been described in Florida and Barbados (Jones et al., 1998; Ward and O'Garro, 1992). These race shifts have been suggested to occur in response to selection by use of resistant cultivars and through copper sensitivity of the races (Pohronezny et al., 1992). Jones et al. (1998) showed sudden shifts from race T1 to race T3 throughout the growing season.

I-2.10 Crop Losses

Losses due to bacterial spot have been attributed to reduced plant vigor and impaired fruit quality, ranging from lesions to sunscald (Cox, 1966). Cox (1966) estimated that annual losses due to bacterial spot in tomatoes in South Florida ranged from 10-100%. Early infection increased the amount of yield loss in tomato up to 20% of total marketable yield. High losses occurred in the USDA large-size category, which brings a premium price to the producer (Pohronezny and Volin, 1983). An estimated $13,080/acre was lost to early infection, based on 1978 prices.

I-2.11 Control of Bacterial Diseases

Effective control of bacterial diseases must be based on knowledge of the life cycle of the pathogen and the disease cycle (Lozano and Wholey, 1974). Cultural

practices are the most efficient and effective way to control bacterial diseases. These practices include adjusting plant density and spacing; rate, timing and number of fertilizer applications; cropping systems; field sanitation; seed health and quality; resistant cultivars; water management; and most importantly, the use of pathogen-free seed and transplants. Burning of plant material after harvest is widely used to reduce initial inoculum levels of the pathogen. This practice kills both epiphytic and endophytic bacteria. Working in fields when climatic conditions do not favor the pathogen, i.e. when plants are dry and relative humidity is low, reduces the spread of the pathogen.

Until the early 1950s, chemical control methods for bacterial diseases were limited (Cox, 1966). The availability of antibiotics for control of bacterial diseases changed this. Use of streptomycin and copper + mancozeb was recommended at this time for control of bacterial diseases. Copper + mancozeb remain the industry standard today. Mancozeb, a fungicide, improves the efficacy of copper in controlling foliar bacterial pathogens.

The rise of antibiotic resistance has since led to the reduced use of antibiotics for disease control in commercial tomato production. Resistance to copper and zinc has also become an increasing problem in disease control. A survey in Barbados showed that 61% of the isolates collected were resistant to copper, 64% were resistant to zinc, and 47% were resistant to streptomycin (Ward and O'Garro, 1992). Copper resistance was first reported in Virginia in 1998 (Alexander et al., 1999).

Seed certification programs are sometimes used to aid in production of bacterial spot/speck-free transplants. Seed treatments consist of hot-water treatments in which seed is soaked in 50 to 58^0C for 20 to 30 minutes (Swings and Civerolo, 1993). The

treatment is not uniform and it adversely affects seed germination and seedling vigor. Other treatments include dry heat and chemical treatments. In dry heat treatment, seed is placed at 71 to 72^0C for 7 days and germination is not affected (Fang et al. 1957). Chemical seed treatments include calcium hypochlorite (Shultz et al., 1986), nyolate (Harman et al., 1987) and acidified cupric acetate (Schaad et al., 1980). Antibiotics, such as streptocycline and streptomycin, were also used as seed treatments until the bacterium became resistant to the antibiotics (Swings and Civerolo, 1993).

Once disease occurs in the field, a bactericide is needed for control. The most widely used bactericides are copper-based and include copper oxychloride, copper hydroxide, cuprous oxide and Bordeaux mixture (Swings and Civerolo, 1993). However, copper resistance is well known to reduce copper efficacy (Ritchie and Dittapongpitch, 1991). Marco and Stall (1983) found that adding mancozeb to copper-resistant or -tolerant isolates increases control. For many years, reports in Fungicide and Nematicide Tests have shown that copper + mancozeb provide the best control of bacterial diseases in many different tomato growing regions.

I-2.12 Systemic Acquired Resistance

The newest control method for bacterial pathogens is Systemic Acquired Resistance (SAR). Ross (1961) coined the term "systemic acquired resistance" (SAR) to describe long-lasting resistance induced by a pathogen to subsequent infections. Compounds that induce SAR enhance the host's own defenses against the pathogen by way of the "same signal transduction pathway as pathogen inoculation" (Anonymous, 1997).

Systemic resistance was first described in 1901 when plants were observed to become resistant to subsequent pathogen inoculations after an initial infection (Ray, 1901). SAR can be induced by many factors. Fungi, bacteria, viruses, nematodes, or metabolites of pathogens, as well as feeding from herbivores, can induce SAR (Dietrich et al., 1999). Potato plants exposed to incompatible isolates of *Phytophthora infestans* were protected from compatible isolates of *P. infestans* (Ozeretskovskaya, 1995). Disease development in response to *Fusarium oxysporum* f.sp. *lycopersici* in tomato plants inoculated with the mycorrhiza-producing fungus, *Glomus mosseae*, was limited compared to non-inoculated plants (Ozeretskovskaya, 1995). Amemiya (1985, 1986) showed that a macromolecular fraction of culture filtrates of non-pathogenic *Verticillium* spp. and *Fusarium* spp. induced resistance of tomato seedlings to pathogenic *Verticillium albo-atrum* and *F. oxysporum* f.sp. *lycopersici* (Amemiya, 1985, 1986). Field tests, which used tomato seeds pre-treated with a biogenic elicitor, enhanced resistance of tomato to *P. infestans, Alternaria solani, Septoria lycopersici, Xanthomonas vesicatoria,* and *Melodigyne incognita* (Ivanyuk et al., 1990).

Many plant pathogen interactions that induce resistance under laboratory conditions are difficult to reproduce in the field. Salicylic acid can induce resistance but is phytotoxic to most crops. Historically, SAR compounds have provided poor control of plant diseases when compared to pesticide applications. SAR induction usually involves complex molecules that are expensive to manufacture and have low field durability (Tally et al., 1999).

One SAR-inducing compound is acibenzolar-S-methyl (acibenzolar). Acibenzolar is a compound in the benzothiadiazole chemical class, that complies with the

definition of a SAR-inducer. To be a SAR-inducing compound, the compound must elicit protection to the same spectrum of pathogens as biological inducers, cause the expression of the same biochemical markers as biological inducers, and not have any anti-microbial activity (Kessman et al., 1994).

Ritchie et al. (1997) showed that acibenzolar provided significantly better control than the industry standard of copper + mancozeb. Louws et al. (2001) compiled data from all over the East Coast on the use of acibenzolar to control bacterial spot and speck of tomato. Disease control was similar or superior to the industry-standard-bactericide programs. There was no evidence of phytotoxicity to the tomatoes in any of the experiments discussed by Louws et al.

Acibenzolar is currently marketed in the U. S. as Actigard™ (Syngenta Crop Protection, Greensboro, N.C.) Its chemical name is benzo (1,2,3) thiadiazole-7-carbothioc acid-S-methyl. It is formulated as a 50% water dispersible granule (Anonymous, 1997). Actigard™'s mode of action mimics the SAR response found in most plant species. Actigard™ is transported systemically and translocated throughout the plant and interferes with the pathogen life cycle at several different sites (syngenta.com). It received registration for use on tomatoes in Virginia in August 2001. Its structure is similar to salicylic acid and it activates the salicylic acid pathway of resistance. In addition, it may activate other pathways against different pathogens (Tally et al., 1999). The induction time of acibenzolar is thought to be 2 to 4 days, so acibenzolar must be used as a preventative means of disease control. During SAR expression, several mechanisms appear to be activated simultaneously against pathogen

establishment. This may reduce the risk of developing SAR-insensitive strains of the bacterial pathogens (Tally et al., 1999).

Table I-1 Toxicity of Pesticides Used on Staked Tomatoes

Pesticide	Acute Oral LD 50 Rat	LC 50 Bluegill	LC 50 *Daphia magna*
Copper Hydroxide	1,346 mg/kg	180.0 ppm	6.5 ppb
Acibenzolar	>5,000 mg/kg	1.6 ppm	2.9 ppm
Chlorothalonil	>5,000 mg/kg	62.0 ppb	70.0 ppb
Azoxystrobin	>5,000 mg/kg	1.1 ppm	259.0 ppb

I-3 Fungal Pathogens

I-3.1 Important Fungal Pathogens

The most important fungal pathogen of tomatoes in Virginia is *Alternaria solani* (*A. solani*), which causes early blight. *Septoria lycopersici* causes Septoria leaf spot, a minor disease in tomatoes on the Eastern Shore of Virginia. The main focus of this section will be on early blight; however, Septoria leaf spot is also included in this research because, like early blight, it can be adequately forecasted by *Tomcast*.

I-3.2 Early Blight

A. solani, which causes early blight, is a pathogen of potato and tomato wherever these crops are grown. Early blight is widespread throughout the tropics and temperate zones (Ellis and Gibson, 1975). The disease is more severe in warmer climates. It also is a pathogen on peppers, eggplants and some other species of *Solanaceae* (Rands, 1917a; Neergard, 1945). The pathogen was first isolated and described by Ellis and Martin in

1882 from dying potato leaves and was called *Macrosporium solani*. In 1892 it was also determined to be a tomato pathogen (Walker, 1952). Most researchers prefer to use *A. solani* (E&M) Jones and Grout. Other names used to describe the pathogen have been *Alternaria porri* f.sp. *solani* and *Alternaria dauci* f.sp. *solani* (Neergard, 1945). These names appear in several publications; however, this paper will use the name *A. solani* to refer to the early blight pathogen.

In tomatoes, the pathogen causes symptoms of collar rot on young seedlings. Following a period of relative host-resistance, symptoms appear on aging foliage and fruit. Susceptibility is influenced by plant size and the yield-to-foliage ratio, as well as the age of the tissue (Barratt and Richards, 1944). Wounds from wind-blown sand and other types of mechanical injury increase susceptibility (Moore, 1942). Insect wounds may also increase susceptibility. Several insects in the U. S. have been observed to carry spores of *A. solani*. Punctures made by these insects were found at the centers of lesions caused by *A. solani* on tomatoes (Martin, 1918). Temporary wilting during the day and low-water stress also increase susceptibility of tomato seedlings to early blight (Moore and Thomas, 1943). Plants that have an imbalance in nutrient demand are also predisposed to disease (Horsfall and Dimond, 1957).

I-3.3 Infection

Germination is facilitated by free moisture but has frequently been reported to occur at relative humidities near saturation. Germination under these conditions may be induced by condensed water on the spores (Schein, 1964). Germination can occur at temperatures ranging from 2 to 40°C (Bashi and Rotem, 1974; Waggoner and Parlange,

1975; Pound, 1951). Germ tubes penetrate the epidermis directly or enter the leaf through stomates or wounds. Wetting period, temperature, and inoculum density affect the level of infection. Under optimum conditions, lesions can appear within 1 day after infection. The bulls-eye pattern of the lesions is determined by diurnal fluctuations in temperature, humidity, and radiation.

I-3.4 Disease Cycle

A. solani can live for several years in the soil due to a dark pigmentation of the hyphae that increases the resistance to lysis (Lockwood, 1960). Thick-walled, dark-brown chlamydospores also enable the pathogen to survive and over-season from crop to crop (Basu, 1971). The mycelium of *A. solani* is heartier than its spores and can survive for extended periods under conditions of extreme temperature and relative humidity (Rotem, 1968). Ultraviolet (UV) radiation can be deleterious to spores of *A. solani*. However, inside the plant, the fungus is protected from harmful wavelengths of UV light. Plant debris and seeds are the main vehicles of over-seasoning mycelium. Colonized seeds carry the pathogen overwinter and are an important source of inoculum for the collar rot phase on tomatoes. Survival in debris is conditioned by meteorological, edaphic, and biotic conditions specific to given locations.

Induction of sporulation is dependent on exposure to UV light. Conidiophores are produced during the first wet night. During the following day, light and dryness induce them to produce spores, which emerge during the second wet night (Bashi and Rotem, 1976). Sugars from photosynthesis may play a role in the reduction of sporulation on

actively growing tissues (Cohen and Rotem, 1970). Sporulation is favored by warm temperatures and leaf wetness from rainwater or high humidity.

Spores are mainly dispersed by the wind and occasionally by splashing rain, overhead irrigation, or insects. Dispersal is favored by conditions that favor spore production, such as nighttime weather and amount of blighted foliage. High wind velocity and dryness are the most favorable conditions for dispersal. Most spores are released from early morning to early afternoon (Rotem, 1964).

Most major outbreaks of early blight occur late in the season due to reduced susceptibility in young plants. In most cases, early blight is a multi-cyclic disease. The disease progress curve of an epidemic depends on local weather conditions (Nash and Gardener, 1988).

I-3.5 Crop Losses

Crop losses from early blight results from loss of blighted foliage, along with decreased photosynthesis and increased respiration in apparently healthy tissue. Because physiological changes are difficult to evaluate, crop loss is based on severity of disease. In 1945, crop losses in tomatoes were reported to be 5 to 50% (Neergard, 1945). A severe epidemic in tomatoes in India resulted in a decrease of 78% in fruit yield (Datar and Mayee, 1981).

I-3.6 Disease Forecasting Systems

The life cycle of *A. solani* has been well studied; thus, early blight is a model disease for forecasting. The first simulator for early blight development was EPIDEM, which simulated the disease according to detailed quantitative analysis of all stages of the

pathogen's life cycle (Waggoner and Horsfall, 1969). EPIDEM mimics an epidemic of *A. solani* on potato, hence the name. However, EPIDEM did not consider changes in plant development, and did not take into consideration an analysis of the pathogen's life cycle and the effects of weather on disease development.

Forecasting for early blight in tomato was developed at Pennsylvania State University, and was based on the identification of environmentally favorable periods for disease development (Madden et al., 1978; Pennypacker et al., 1983). This model was named the "FAST model," which stood for Forecasting *Alternaria solani* on Tomatoes. Traditionally, control of the disease is begun with chemicals when the first fruits are set and applications continue at 7- to 10-day intervals, regardless of environmental conditions. The FAST model uses two sub-models to determine periods when environmental conditions are favorable for disease development. One sub-model defines a daily disease severity value according to leaf wetness periods and mean ambient air temperature during the wetness periods. The second sub-model defines a daily disease severity rating based on mean ambient air temperature, hours of relative humidity over 90% for the past 5 days, and total rainfall for the past 7 days (Madden et al., 1978). Under conditions very favorable for disease, the number of recommended sprays could equal or even exceed those applied according to a 7-day schedule. However, in less favorable seasons the number of recommended applications could be fewer (Pennypacker et al., 1983).

Drs. Madden, Pennypacker, MacNab and Pitblado developed the model TOMCAST, derived from the words, tomato and forecast, from the original FAST model. TOMCAST relies on leaf wetness and temperature to determine disease severity and

generates Disease Severity Values (DSVs) as units of disease development for diseases such as early blight and Septoria leaf blight. DSVs provide a numerical representation of the rate at which disease pressure accumulates on tomato plant tissue. The DSVs are determined by two factors: leaf wetness and temperature during the leaf wetness hours. As the number of leaf wetness hours and temperature increases, DSVs increase. Conversely, when there are fewer hours of leaf wetness and the temperature is low, DSVs are reduced. Table I-2 shows the interaction between these factors.

When the total number of accumulated DSVs exceeds a pre-determined limit, namely the spray threshold, a fungicide spray is applied to protect the foliage and fruit from disease development. The exact spray threshold can range from 15 to 25 DSVs. The exact spray threshold a grower should use depends on the anticipated fruit quality and end-use of the tomatoes. By following a 15-DSV-spray threshold, which represents a more conservative use of the TOMCAST system, a grower will apply fungicides more frequently than a grower who uses a 20-DSV-spray threshold. The trade-off is in the number of sprays applied during the season and the potential for differences in fruit quality. A 15-DSV-spray threshold can be roughly translated into a 7- to 10-day spray program, where as a 20-DSV-spray-threshold program translates into a 10-to 14-day-application interval, depending on the weather pattern.

Table I-2 Disease Severity Values (DSVs) Chart: Relationships of DSVs to Determine *Tomcast* Applications

Avg. Temp. During Leaf Wet Hrs	Hrs of Leaf Wetness per Day				
13-17 degree C	0-6	7-15	16-20	21 +	
18-20 degree C	0-3	4-8	9-15	16-22	23+
21-25 degree C	0-2	3-5	6-12	13-20	21+
26-29 degree C	0-3	4-8	9-15	16-22	23+
Daily DSV total	0	1	2	3	4

I-3.7 Septoria Leaf Spot

Septoria leaf spot is caused by the fungus *Septoria lycopersici* (*S. lycopercici*). The fungus reproduces by producing long, filiform, colorless, multi-cellular conidia produced in dark, globose, pycnidia. Pycnidia can produce long tendrils of spores. The conidia are disseminated by rain splash or irrigation water or they can be spread mechanically. *S. lycopercici* overwinters as mycelium, conidia, or pycnidia on or inside colonized seed and plant debris left in the field.

I-3.8 Symptoms and Control of Septoria Leaf Spot

Leaf spots begin as small, yellowish specks that later enlarge and turn pale-brown or yellowish-gray and finally dark-brown. The disease starts on the lower foliage and works its way upward. Lesions range from 1 to 3 mm in diameter (Agrios, 1978).

Control of Septoria leaf spot depends on the use of pathogen-free seed. A 2 to 3-year crop rotation, sanitation by deep plowing of diseased plants, and use of resistant cultivars help to control the disease. Fungicides used for disease control include mancozeb, mancozeb + zinc, chlorothalonil, and Bordeaux mixture (Agrios, 1978).

Azoxystrobin is a new fungicide, which is now being used in Septoria leaf spot control (Alexander et al., 1997).

I-3.9 Control of Fungal Diseases

The most popular control method for early blight and less prevalent fungal diseases of tomato has been application of a protective fungicide in combination with cultural practices that reduce the amount of inoculum. Jones (1892) controlled early blight with Bordeaux mixture. Until recently, chlorothalonil and mancozeb provided the best control of fungal diseases in tomatoes and were recommended for control of early blight (Alexander et al., 1997). Chlorothalonil acts on the enzyme systems in fungi. It persists on the surface of plant foliage. Chlorothalonil is a contact fungicide that acts to prevent fungal diseases in plants (Anonymous, 2001). A recently developed acropetal penetrant fungicide, azoxystrobin, currently provides better control of fungal pathogens than the contact fungicides, chlorothalonil and mancozeb.

Azoxystrobin, the active ingredient in Quadris®, was derived from naturally occurring fungicidal compounds, called strobilurins, in certain species of wood-decaying mushrooms. Strobilurins are produced by various species of mushrooms, including *Strobilurus tenacellus* and *Oudemansiella mucida*. The ability of these mushrooms to produce anti-fungal products helps them to compete with other fungi for nutrients in their ecological setting (Anonymous, 2000).

Azoxystrobin possesses a novel biochemical mode of action that is the same as the naturally occurring strobilurins: inhibition of mitochondrial respiration in fungi. This is achieved by the prevention of electron transfer between cytochrome b and cytochrome

c1 in the biochemical pathway of mitochondrial respiration. Mitochondria are microscopic bodies found outside the nucleus of the fungus cell. They are important to the survival of the fungus because they produce energy (ATP) for the cell through respiration. Without ATP, fungal cells cannot survive (Anonymous, 2000).

I-4 Summary and Objectives

I-4.1 Summary

The Eastern Shore is strongly dominated by production agriculture, with a large emphasis in vegetable production. Of the vegetables grown, fresh-market-staked tomatoes represent the largest crop in both acreage and value. The high volume and value of the crop have led to high inputs for disease control. The use of protectant fungicides and bactericides, which must be applied often and in high amounts, has given rise to environmental concerns. The volume of fungicides and bactericides applied to tomatoes produced in plasticulture has led to water quality issues related to tomato-field run-off. The sandy texture of soils and low organic matter compound the dangers of harm to aquatic life. These concerns are a result of the high toxicity of these chemicals to shellfish and other aquatic organisms where clams are being produced.

In many cases, the pesticides used are not effective. In the case of foliar bacterial diseases of tomato, it is known that when conditions favor disease development, copper + mancozeb do not provide adequate control. Copper can be rendered useless as a control measure when copper-tolerant or copper-resistant strains are present. However, with copper + mancozeb as the only tool for bacterial control, growers unaware of the

presence of resistant bacterial populations will treat their fields more often and at higher rates, thus exacerbating the problem.

In the case of fungal disease control, chlorothalonil as a pollutant is the main concern. Clams are highly sensitive to this chemical, which is applied often and is subject to being washed off of the plant and plastic mulch. Fungal disease problems are a perennial problem on the Eastern Shore and preventative sprays have always provided the best control. However, new tools can be incorporated into the system to reduce chlorothalonil inputs. For example, TOMCAST can be used to reduce the number of applications per crop, and azoxystrobin, which is used at lower rates and is safer for humans and the environment, may replace chlorothalonil. A potential tool for control of foliar bacterial diseases in tomato production is the use of acibenzolar, which induces SAR.

I-4.2 Objectives

The objectives of the studies reported in this thesis were the following: 1) to achieve acceptable control of bacterial diseases with alternatives to copper bactericides in tomato production; 2) to achieve acceptable control of fungal diseases using disease forecasting and chlorothalonil replacements and 3) to reduce the amounts of copper and chlorothalonil in staked tomato production on Virginia's Eastern Shore.

II. Reducing Copper Use in Staked Tomato Production

II. 1 Introduction

Tomato (*Lycopersicon esculentum* Mill.) production on the Eastern Shore of Virginia in 2000 was approximately 1,659 ha and had an estimated value of $30,888,000 (Manheimer and Vick, 2001). In plasticulture production plastic mulch is placed over raised beds with trickle irrigation and a trellis system to support the tomato plants. Plasticulture provides the most efficient way to manage water, nutrients, diseases, weeds and insect pests, and results in yields up to three times higher and 7 to 21 days earlier than in non-plasticulture systems (Aylsworth, 1997). In plasticulture, mulch covers approximately 35% of the field. Because of the impermeability of the plastic, the potential for rainfall run-off is increased. Run-off may carry sediment and crop protection chemicals from the field into other ecosystems, such as estuaries (Garmond, 1994; Scott et al., 1990). Agriculture and aquaculture often exist immediately adjacent to each other and are inextricably connected in fingers of the Chesapeake Bay and the Atlantic Ocean, which intrude into the agro-ecosystem of the Eastern Shore of Virginia. Recent data collected from some estuaries showed copper at levels potentially toxic to clams and other inhabitants of these estuaries (Dietrich et al., 1996; Luckenbach et al., 1996).

Copper + mancozeb are the primary pesticides used to control bacterial diseases on tomato. Bacterial diseases are difficult to control and can be a limiting factor in tomato production in Virginia. Bacterial spot of tomato, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is extremely difficult to control under moist, warm conditions, which can occur on the Eastern Shore of Virginia. When the weather

conditions become moist and cooler than normal, bacterial speck, caused by *Pseudomonas syringae* pv. *tomato* (Okabe 1933) Young, Dye & Wilkie 1978, becomes the predominant bacterial disease. Bacterial disease development is favored by temperatures ranging from 24 to 30^{o}C, high rainfall, and low-pressure disturbances with high wind velocity. The bacteria penetrate plant tissue through wounds created by wind-driven sand, insects or mechanical disturbances, such as stringing, pruning and harvesting (Vakili, 1967). Severe defoliation and fruit spotting result in significant yield losses. Three races of *X. campestris* pv. *vesicatoria* (T1, T2 and T3) have been described and two have been identified on the Eastern Shore (T1 and T3) (Jeff Jones, personal communication).

Copper + mancozeb provides adequate control when environmental conditions are marginal for disease development, but becomes considerably less effective when environmental conditions favor disease development and inoculum levels are high (Jones et. al. 1986). Current control recommendations for bacterial diseases include 1.21 kg a.i./ha of fixed copper plus 1.68 kg a.i./ha of mancozeb applied on a 5- to 7-day schedule (Alexander et al. 2000). Approximately 51,408 kg of copper were applied in the year 2000 in an effort to control bacterial diseases on the Eastern Shore of Virginia. With the presence of copper-resistant strains of these bacterial pathogens, copper-based control measures have become less effective (Alexander et al., 1999).

Control of bacterial diseases is essential for tomato production to be profitable. In an environmentally sensitive area, such as the Eastern Shore of Virginia, the most effective way to reduce pesticide run-off from plasticulture is to reduce the amount of pesticides applied for disease management. The objective of this study was to determine

the efficacy of acibenzolar-S-methyl as a replacement for copper-based pesticides for the control of bacterial spot and bacterial speck diseases in tomato production on the Eastern Shore of Virginia.

II-2 Materials and Methods

Experiments were carried out in commercial plasticulture tomato fields located in Bloxom, Va. (98A); Modestown, Va. (99A); Painter, Va. (99B); Parksley, Va. (00A) and Tasley, Va. (00B) on the Eastern Shore during the summers of 1998, 1999 and 2000. Experiments were also carried out at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, Va. (98B and 99C). In commercial fields experiments were initiated after bacterial disease was observed in the field. At the ESAREC experiments were initiated prior to disease development.

General field preparations for the experiments at the ESAREC were the same as for commercial tomato production, beginning with moldboard plowing of a rye cover crop in the early spring. Soil was further mixed and leveled by field cultivation. Fertilizers were applied and incorporated with a field cultivator or through trickle irrigation systems. Beds were formed with a soil bed raiser. Trickle irrigation tubing was laid at the same time that methyl bromide (45 kg/ha) was applied, then the beds were covered with plastic mulch and sealed for several weeks prior to planting. During this time the irrigation system was attached to the water source. Transplants were planted with a single row transplanter. The rows were then staked at a 45-cm spacing and plants were trellised with string 3 to 4 times per season as they grew.

The experiments were laid out in randomized block designs with four replicates per treatment. The experiments were maintained with conventional fertilization, weed and insect control. Experiments 98A, 99A and 99B contained plots with single rows, 4.6 m long, spaced 1.8 m apart, with plant spacing of 0.6 m. Experiment 99C had single row plots 9.2 m long, spaced 1.8 m apart, with a plant spacing of 0.5 m. Treatments were initiated when plants were 30-61 cm high (pre-bloom). Experiments conducted in commercial fields in 2000 (00A & 00B) had single-row plots 3.0 m long, spaced 1.8 m apart, with a plant spacing of 0.6 m. Table II-2.1 shows transplanting and harvesting dates for all experiments.

Treatments were reapplied in all tests on a 7- to 10-day interval. Date of application, relative humidity, cloud cover and growth stages at time of treatment applications are found in Tables II-2.2-2.6. Names, formulations and rates for all treatments are shown in Table II-2.6. Treatments for experiments 98A, 99A, 99B, 00A and 00B were applied with a propane-pressurized backpack sprayer, which delivered 486 liters/ha at 276 kPa. The spray boom consisted of three nozzles with one nozzle in the center and the two outer nozzles on 22.5 cm-drop pipes. The distance from the center nozzle to the drop pipes was 50 cm. Each nozzle contained a 50-mesh stainless steel strainer, D4 brass discs and #45 brass cores (TeeJet Spraying Systems Co). The boom was held parallel to the staked tomato row with one of the drop nozzles positioned over the top of the tomato plants, the center nozzle directed toward the center of the plants and the other drop nozzle directed toward the bottom of the plants. Both sides of the tomato rows were sprayed.

Experiment 99C was sprayed with a multi-boom sprayer mounted on a high-clearance Hagie™ tractor that delivered 373.5 liters/ha at 276 kPa. The spray boom consisted of five nozzles with one nozzle positioned over the row and two nozzles, each on two drop pipes 90 cm from the center nozzle. The distance from the first drop nozzle to the top of the boom was 60 cm and the distance to the second drop nozzle was 90 cm. Each nozzle contained a 50-mesh stainless steel strainer and a hollow cone TXVS-18 spray tip (TeeJet Spraying Systems Co).

II-2.1 Ratings

Percent foliar bacterial infection was estimated using the Horsfall-Barrett visual rating acuity scale (Horsfall and Barratt, 1945). Research sites in commercial fields were selected based on uniformity of infection. Initial assessment of foliar infection was taken before any treatments were applied. Disease ratings were taken three times in 1998, three times in 1999 and four times in 2000.

Fruit was harvested from six plants per plot in experiments 98A and 99C. Fruit was harvested from four plants per plot in both commercial plantings in experiments 99A and 99B. Two plants were harvested per plot in experiments 00A and 00B. Fruits were sized and graded, and separated into two categories: those with < 5 and those with >5 bacterial spots per fruit. Data were collected on the number of fruit and total weight for each category.

Bacterial isolates were taken from leaves and fruit in each experiment. Leaves were washed for 20 minutes in running water with several drops of Tween 20, then dipped in a 0.525% NaOCl for 15 seconds, followed by two 15-second washes in sterile

distilled water. Sections of leaf tissue were transferred to approximately 3 ml sterile saline (0.85%) in a sterile petri plate, covered and allowed to sit for 1 hour, then streaked with an inoculating loop onto tryptic soy agar (TSA) in 9 cm diameter petri plates (Difco). After 48 hours of incubation at 27^{o}C, bacteria from single isolated colonies were transferred to blood agar plates (Gibson Laboratories, Inc., KY), which contained 5% sheep's blood in TSA. Bacteria were then allowed to grow for 24 hours before being identified. Bacteria were identified using the Biolog Microlog^{TM} System (Biolog, Inc., CA). Bacteria positively identified as *P. syringae* pv. *tomato* (1998) or *X. campestris* pv. *vesicatoria* (1999) were sent to Dr. Seong Hwan Kim, Pennsylvania Department of Agriculture, for confirmation of identification and copper-sensitivity testing.

Table II-2.1 Cultivars, and transplant and harvest dates for all experiments.

Experiment	*Tomato cultivar*	*Date of transplanting*	*Harvest dates*
98A	Sunpride	April 12	July 7 and 21
98B	Sunbeam	May 18	July 22 and August 10
99A	Solarset	June 26	September 3
99B	Roma BHN 411	July 3	September 22 and October 6
99C	Sunbeam	May 27	July 30; August 12, 27; September 8
00A	Sunpride	April 24	July 18 and August 7
00B	Sunpride	April 27	July 19 and August 3

Table II-2.2 Date, temperature, relative humidity, cloud cover and growth stage at the time of treatment applications for experiment 98A.

98A

Date	*Temperature ºC*	*% Relative humidity*	*Wind speed km/hr*	*% Cloud cover*	*Growth stage*
5/19	28.3	49	6.4-9.6	0	Pre-bloom
5/26	26.1	59	9.6-12.8	0	Early bloom
6/2	24.4	57	8.0-11.2	0	Full bloom
6/9	23.3	43	3.2-6.4	60	Early fruit set
6/16	28.3	59	11.2-14.5	0	Fruit set
6/26	29.4	73	0-3.2	0	Green-fruit
7/2	26.7	53	6.4-11.2	0	Mature green fruit
7/10	28.9	66	11.2-16.0	0	Post-harvest
7/17	30.0	70	4.8-8.0	30	Mature green
7/24	29.4	55	8.0-11.2	0	Mature green

Table II-2.3 Date, temperature, relative humidity, cloud cover and growth stage at the time of treatment applications for experiments 99A and 99B.

99A

Date	*Temperature ºC*	*% Relative humidity*	*Wind speed km/hr*	*% Cloud cover*	*Growth stage*
7/28	32.7	50	9.6-12.8	0	Pre-bloom Plants 61cm
8/4	23.3	84	6.4-9.6	0	Early bloom
8/11	30.0	59	8.0-11.2	100	Full bloom
8/18	31.1	70	3.2-6.4	20	Early fruit
9/8	28.9	77	3.2-6.4	0	Mature green fruit
9/21	24.4	71	0-3.2	100	Mature green fruit

99B

7/27	32.2	48	9.6-12.8	80	Pre-bloom 30 cm
8/3	28.3	66	9.6-16.0	0	Pre-bloom
8/10	28.9	60	0	0	Bloom
8/17	28.9	82	8.0-16.0	0	Early fruit
8/24	27.2	71	0	0	Immature green fruit
9/1	21.1	67	6.4-11.2	100	Immature green fruit
9/13	25.0	71	1.6-4.8	100	Mature green fruit
9/24	21.1	57	4.8-9.6	0	Mature green fruit
10/1	18.9	53	0	0	Mature green fruit

Table II-2.4 Date, temperature, relative humidity, cloud cover and growth stage at the time of treatment applications for experiment 99C.

Date	*Temperature ^{o}C*	*% Relative humidity*	*Wind speed km/hr*	*% Cloud cover*	*Growth stage*
6/25	24.4	72	0-3.2	0	Pre-bloom 30-45 cm
7/2	26.7	68	8.0-14.5	0	Early bloom
7/8	32.2	36	3.2-6.4	0	Full bloom
7/15	25.6	66	4.8-6.4	20	Early fruit
7/21	26.1	75	12.8-16.0	100	Early fruit
7/26	30.0	57	9.6-12.8	40	Mature green fruit
8/4	26.7	54	6.4-8.0	0	Mature green fruit
8/11	30.0	59	8.0-12.8	100	Mature green fruit
8/24	27.2	55	8.0-11.2	0	Mature green fruit

Table II-2.5 Date, temperature, relative humidity, cloud cover and growth stage at the time of treatment applications for experiments 00A and 00B.

00A

Date	*Temperature ºC*	*% Relative humidity*	*Wind speed hm/hr*	*% Cloud cover*	*Growth stage*
6/28	26.1	77	3.2-6.4	100	Early fruit-set
7/6	27.2	60	3.2-8.0	0	Fruit-set
7/12	26.1	57	4.8-8.0	50	Immature green fruit
7/18	31.7	44	3.2-9.6	40	Mature green fruit
7/26	27.8	70	0-4.8	100	Mature green fruit

00B

6/28	26.1	77	3.2-6.4	100	Early fruit-set
7/6	27.2	60	3.2-8.0	0	Fruit-set
7/12	26.1	57	4.8-8.0	50	Immature green fruit
7/19	31.1	60	0-3.2	100	Mature green fruit
7/28	27.8	68	3.2-8.0	100	Mature green fruit

Table II-2.6 Treatment information for all experiments, including trade name, formulation and rate.

Treatments	**1998-2000**		
Common Names	**Trade name**	**Formulation**	**Rate** a.i./hectare
Acibenzolar	Actigard®	50 W	10.5 g
Acibenzolar +	Actigard® +	50 W	10.5 g
Copper hydroxide	Kocide 2000®	77 DF	1.21 kg
Acibenzolar +	Actigard® +	50 W	10.5 g
Azoxystrobin	Quadris®	2 SC	0.1 kg
Mancozeb +	Dithane® +	75 DF	1.68 kg
Copper hydroxide	Kocide 2000®	77 DF	1.21 kg
Mancozeb	Dithane®	75 DF	1.68 kg
Chlorothalonil +	Bravo WS®	6 F	1.68 kg
Copper Hydroxide	Champ formula 2®	4.6 F	0.83 kg
Chlorothalonil	Bravo WS®	6 F	1.68 kg
Untreated control	-	-	-

II-3 Results

II-3.1 Control of Bacterial Speck

Acibenzolar applied at 10.5 g a.i./ha significantly reduced the amount of leaf area affected by *Pseudomonas syringae* pv. *tomato* compared to the non-treated control as well as the standard treatment (copper hydroxide + mancozeb) in all experiments (Table II-3.1). In experiment 98A, acibenzolar provided significantly ($P \leq 0.05$) better foliar disease control than chlorothalonil or chlorothalonil + copper. In experiments 20A and 20B, acibenzolar plus azoxystrobin or copper hydroxide provided significantly ($P \leq 0.05$) better control than the standard treatment. The addition of copper hydroxide or azoxystrobin to acibenzolar did not significantly ($P \leq 0.05$) increase the level of foliar disease control as compared to acibenzolar alone. Chlorothalonil, chlorothalonil + copper and mancozeb alone provided significantly ($P \leq 0.05$) better foliar disease control than the untreated control in all studies except for chlorothalonil + copper in study 98A.

In 1998, acibenzolar provided slightly better protection against fruit infection and, therefore, higher marketable yield than the standard treatment (Table II-3.3). In the acibenzolar treatment in 98A, plants produced 2.7 tons/ha more marketable fruit than in the standard treatment. Chlorothalonil alone and in combination with copper hydroxide resulted in the smallest marketable yield. In experiments 00A and 00B, there were no significant ($P \leq 0.05$) differences in marketable yields among treatments. However, in experiment 00A the marketable yield from acibenzolar alone was 8.1 tons/ha more than for the standard. In 00B the standard treatment resulted in 8 tons/ha more marketable fruit.

II-3.2 Control of Bacterial Spot

Acibenzolar provided significantly ($P \leq 0.05$) better foliar bacterial spot protection than the untreated control (Table II-3.2). The acibenzolar treatment alone did not significantly ($P \leq 0.05$) differ from the standard in percent disease severity, although numerically acibenzolar averaged 15% better control. In experiment 99B, the acibenzolar + copper treatment provided significantly ($P \leq 0.05$) better control than the standard treatment. The acibenzolar treatment with copper or azoxystrobin in experiment 99C provided significantly ($P \leq 0.05$) better control than the standard treatment. In experiment 99B, only acibenzolar and acibenzolar + copper provided significantly ($P \leq 0.05$) better disease control than the non-treated control. The standard treatment was not statistically ($P \leq 0.05$) different from the non-treatedcontrol for experiment 99B. In experiments 99A and 99C, the standard treatment provided significantly ($P \leq 0.05$) better protection from foliar infection than the control. In experiment 99A, mancozeb and chlorothalonil + copper provided disease control significantly ($P \leq 0.05$) better than the control.

The acibenzolar treatments were not significantly ($P \leq 0.05$) different from the standard treatment (Table II-3.3). In experiment 99A, the acibenzolar + copper hydroxide, acibenzolar + azoxystrobin, and chlorothalonil treatments resulted in statistically ($P \leq 0.05$) higher marketable yields than the control. The chlorothalonil + copper treatment, which resulted in a yield of 65.2 tons/ha, was the only treatment that showed statistically ($P \leq 0.05$) greater yields than the control in experiment 99B; however, the marketable yield for the chlorothalonil treatment was not statistically ($P \leq$

0.05) different from that of the other treatments. In experiment 99C, there were no significant ($P \leq 0.05$) differences in marketable yields among treatments.

In 1998, a field isolate of *P.s.* pv. *tomato* was determined to be tolerant to high levels of copper where the treatment with copper alone was not as effective as the control (Alexander et al., 1999). The isolate was inhibited by 368 µg/ml copper hydroxide as compared to a sensitive isolate that was inhibited by 175 µg/ml copper hydroxide. Growth of a field isolate (1999) of *X. c.* pv. *vesicatoria* was inhibited at a concentration of 406 µg/ml copper hydroxide as compared to a copper-sensitive isolate that was inhibited at 88 µg/ml copper hydroxide.

Table II-3.1 Control of bacterial speck foliar infection on staked tomatoes by acibenzolar and standard bactericides with all treatments applied on a 7-day application interval in 1998 and 2000.

	Disease Severity[1]		
Treatment	98A	20A	20B
Acibenzolar	6.3 c[2]	21.0 d	20.0 d
Acibenzolar + Copper hydroxide	-----	21.3 d	20.0 d
Acibenzolar + Azoxystrobin	-----	21.8 d	20.3 d
Mancozeb + Copper hydroxide	16.3 b	31.5 c	22.8 c
Mancozeb	-----	29.5 c	26.3 b
Chlorothalonil + Copper hydroxide	25.0 a	32.5 bc	25.8 b
Chlorothalonil	17.0 b	36.8 b	27.0 b
Untreated control	24.5 a	48.3 a	33.5 a
LSD	4.97	4.79	1.41

[1] Disease severity is a rating of leaf area affected (Horsfall and Barratt, 1945)

[2] Values followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Duncan's new multiple range test.

Table II-3.2 Control of bacterial spot foliar infection on staked tomatoes by acibenzolar and standard bactericides in 1999.

	Disease Severity[1]		
Treatment	99A	99B	99C[3]
Acibenzolar	30.0 c[2]	28.8 bc	22.5 cd
Acibenzolar + Copper hydroxide	32.5 bc	17.5 c	17.5 d
Acibenzolar + Azoxystrobin[3]	32.5 bc	33.8 ab	17.5 d
Mancozeb + Copper hydroxide	36.3 bc	32.5 ab	27.5 bc
Mancozeb	42.5 b	35.0 ab	32.5 b
Chlorothalonil + Copper hydroxide	41.3 b	38.8 ab	28.8 bc
Chlorothalonil	53.8 a	------	31.3 bc
Untreated control	55.0 a	45.0 a	50.0 a
LSD	10.60	12.54	9.68

[1] Disease severity is a rating of leaf area affected (Horsfall and Barratt, 1945).

[2] Values followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Duncan's new multiple range test.

[3] Azoxystrobin in the 99C study was applied four times according to *Tomcast*, 25DSV and 15DSV, with acibenzolar applied on the last three applications after detection of bacterial spot. All other treatments = 7-day application interval.

Table II-3.3 Effects of acibenzolar and standard fungicide applications on marketable yield (fruit with no bacterial infection) in staked tomatoes grown on the Eastern Shore of Virginia in 1998-2000.

	Marketable Yield (t/ha)					
	X. campestris* pv. *vesicatoria			***P. syringae* pv. *tomato***		
Treatment	**99A**	**99B**	**99C[2]**	**98A**	**00A**	**00B**
Acibenzolar	43.7 abc[1]	48.9 ab	65.5 ab	28.3 a	46.9 a	50.3 a
Acibenzolar + Copper hydroxide	51.3 ab	62.5 ab	70.3 ab	-----	38.0 a	58.4 a
Acibenzolar + Azoxystrobin[2]	49.7 ab	58.4 ab	72.5 a	-----	40.4 a	58.4 a
Mancozeb + Copper hydroxide	41.9 abc	58.3 ab	65.9 ab	25.6 a	38.8 a	58.3 a
Mancozeb	45.9 abc	60.3 ab	70.1 ab	-----	41.3 a	58.9 a
Chlorothalonil + Copper hydroxide	36.2 bc	65.2 a	63.0 b	20.1 a	41.3 a	53.8 a
Chlorothalonil	52.3 a	-----	72.7 a	20.7 a	41.0 a	55.0 a
Untreated control	31.8 c	45.6 b	65.3 ab	24.9 a	36.0 a	59.8 a
LSD	13.8	17.0	7.8	10.5	11.1	10.2

[1] Values followed by the same letter are not significantly different according to Duncan's new multiple range test ($P \leq 0.05$).

[2] Azoxystrobin in the 99C study was applied four times according to Tomcast, 25DSV+15DSV, with acibenzolar applied on the last three applications after detection of bacterial spot. All other treatments = 7-day application interval.

II-4 Discussion

Controlling diseases, reducing pesticide usage and addressing environmental concerns are important issues in modern agriculture. These factors all come to the forefront in a high value crop, such as fresh market tomatoes produced on the unique ecosystem of Virginia's Eastern Shore. Copper has been the best production tool available for managing foliar bacterial pathogens for more than 40 years. However, standard copper bactericides cannot provide adequate control of foliar bacterial diseases in tomatoes when conditions are highly favorable for disease development (Jones et al., 1986). The existence of copper-tolerant and/or copper-resistant populations further complicates foliar bacterial disease control. Using copper often and at high rates to control bacterial spot and bacterial speck has been a practice used by producers on the Eastern Shore because no other effective alternatives were available.

The ecosystem of the Eastern Shore is such that tomato production fields are within meters of estuaries where clams are produced. In 1996, losses in clam nurseries were thought to be associated with pesticide run-off from commercial tomato production areas. The pesticides causing the most concern contained copper, which is toxic to clams at relatively low levels (Dietrich et al., 1996). The objective of this study was to identify and evaluate a replacement for copper pesticides that was safer for the environment and could provide at least comparable control of the bacterial diseases of tomato.

Acibenzolar-S-methyl is not a fungicide/bactericide in the usual sense; in fact, it is not a 'cide' at all. Instead it triggers plants to activate their own natural defenses through a process called Systemic Activated Resistance (SAR). In nature, SAR is

sporadic, but by treating plants with acibenzolar, this "natural" response produces a more consistent level of protection against many fungal and bacterial pathogens of plants.

Acibenzolar effectively controlled bacterial speck and bacterial spot on the Eastern Shore of Virginia. Acibenzolar, tank-mixed with a fungicide or bactericide, did not provide better control of bacterial diseases when compared to acibenzolar alone in any of the studies. Acibenzolar did not cause a reduction of yield in any of the six experiments. For control of bacterial speck, acibenzolar and tank-mixes with a fungicide were superior to the standard treatment, copper hydroxide + mancozeb. In the three 1999 experiments, acibenzolar and tank mixes containing acibenzolar provided equivalent or superior control of bacterial spot when compared to the standard treatment.

The use of growers' fields provided a unique opportunity to determine the practical uses and efficacy of acibenzolar. In comparison to the standard treatment, acibenzolar provided better control of foliar infections of *P. syringae* pv. *tomato,* but acibenzolar was some what less effective against foliar infections of *X. campestris* pv. *vesicatoria*. The addition of copper to acibenzolar may be more effective in managing bacterial spot than acibenzolar alone. A combination treatment would still represent a major reduction in the amount of copper used to manage bacterial spot. Also, evidence from the 1998 experiment showed that acibenzolar may provide better protection against fruit infection by bacterial speck as suggested by the high amount of marketable yield. The differences in disease severity may be a result of differing levels of copper sensitivity or simply differing environmental and cropping conditions for each growing season. Among all three growing seasons and six fields, the best control resulted from the use of acibenzolar or tank mixes of fungicides in combination with acibenzolar.

Early field evaluations of acibenzolar in fresh market tomato production showed signs of phytotoxicity and yield reductions (personal communications, Gary Schnappinger, Syngenta). The rates used in these field evaluations were 30 g/ha and higher. In our experiments, there were no signs of phytotoxicity or yield loss from any of the acibenzolar treatments at a rate of 10.5 g/ha.

The existence of tomato fields in close proximity to aquaculture production supports the use of a compound, such as acibenzolar –S-methyl. Acibenzolar is used at low rates (10.5 – 31.5 g/ha per application), compared to the standard (copper hydroxide 1.21 kg/ha + mancozeb 1.68 kg/ha per application). Acibenzolar also has better decomposition properties than copper. Acibenzolar, when applied to soil, has not been found below the three-inch depth and has a half-life of less than one day in loamy soils (Anonymous, 1997). The use of acibenzolar can reduce pesticide inputs dramatically. An average of 17 applications of copper + mancozeb are made per crop with current standard control treatments. On 1,659 ha, 47,877 kg of copper hydroxide + mancozeb are applied annually. This puts the total pesticide input for foliar bacterial control to 95,754 kg/yr. On the same hectareage and at the maximum amount of 221 g/ha/crop, the total amount of acibenzolar would be 367 kg/ha/yr, a 99.6% reduction in the volume of pesticide inputs.

The existence of copper-resistant strains of the pathogens further favors the use of acibenzolar. During SAR expression several mechanisms appear to be activated simultaneously against a pathogen attack, although there is no direct activity against the pathogen. The risk of a pathogen developing SAR-insensitive strains is likely reduced. However, pathogens have overcome host defenses in the past, so a disease management

program should be maintained to further reduce the likelihood of development of insensitivity to acibenzolar.

The results of this study support the findings reported by Louwes et al. (2001) favoring the use of acibenzolar over copper; however, the effective rate of 10.5g/ha is lower in this study than reported by Louwes et al. Acibenzolar addresses the concerns of both producers and the seafood industry on Virginia's Eastern Shore. The use of acibenzolar to control bacterial diseases of tomatoes on the Eastern Shore is an essential tool for meeting the objectives of providing good disease control and environmental compatibility within a sensitive ecosystem through low application rates and a short residual life. The results of this study show that acibenzolar can provide producers on the Eastern Shore with an alternative to copper that is better for the ecosystem and is a better tool for managing bacterial spot and bacterial speck in fresh market tomato production.

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III. Reducing Chlorothalonil Inputs by Using Disease Forecasting and Azoxystrobin for Control of Fungal Pathogens

III-1 Introduction

On the Eastern Shore of Virginia in the 2000 growing season, tomatoes (*Lycopersicon esculentum* Mill.) were produced on approximately 1,579 ha and had an estimated value of $30,888,000 (Manheimer and Vick, 2001). In the plasticulture production system, plastic mulch is placed over raised beds with trickle irrigation and a trellis system supporting the tomato plants. Plasticulture provides the most efficient way to manage water, nutrients, diseases, weeds and insect pests, and results in yields up to three times higher and 7 to 21 days earlier than in non-plasticulture systems (Aylsworth, 1997). In plasticulture, mulch covers approximately 35% of the field. Because of its impermeability, the plastic increases the potential for rainfall run-off. Run-off may carry sediment and crop protection chemicals from the field into other ecosystems, such as estuaries (Garmond, 1994; Scott et al., 1990). Agriculture and aquaculture often exist adjacent to each other and are inextricably connected in fingers of the Chesapeake Bay and the Atlantic Ocean, which are interwoven into the agroecosystem of the Eastern Shore of Virginia. Recent studies have shown the presence of chlorothalonil in these estuaries (Dietrich et al., 1996; Luckenbach et al., 1996).

The most important fungal pathogen in Virginia on tomatoes is *Alternaria solani*, which causes early blight. *Septoria lycopersici*, which causes Septoria leaf spot, is a minor disease that can become a problem when conditions are favorable for its development. Until recently, chlorothalonil and mancozeb were recommended as the best fungicides available for control of early blight and other fungal diseases of fresh market tomatoes (Alexander et al., 1997). Currently an acropetal penetrant fungicide,

azoxystrobin, is available and provides better control of fungal leaf diseases. Azoxystrobin has a half-life in soil of less than one day. Because of concern over fungicide and bactericide levels in run-off into estuaries of the Chesapeake Bay and the Atlantic Ocean, the short half-life of this fungicide would be beneficial to the tomato producers and the aquaculture industry of the Eastern Shore.

Another way to reduce fungicide inputs is disease forecasting. The first simulator for forecasting disease development was EPIDEM, which simulated disease according to detailed quantitative analysis of all stages of the pathogen's life cycle (Waggoner and Horsfall, 1969). Because of its well-documented disease cycle, early blight is a model disease for which forecast systems can be used for control.

Forecasting for early blight in tomato, developed in Pennsylvania, was based on the identification of environmentally favorable periods for disease development (Madden et al., 1978; Pennypacker et al., 1983). This system was named the "FAST model," an acronym for forecasting *Alternaria solani* on tomatoes. The FAST model was based on the tradition that control of the disease is started when the first fruits are set and continued at a 7- to 10-day intervals, regardless of environmental conditions. It uses two sub-models to determine periods when environmental conditions are favorable for disease development. One sub-model defines a daily disease severity value, according to leaf wetness periods, and mean ambient air temperature during the wetness period. The second sub-model defines the daily disease severity rating based on mean ambient air temperature, hours of relative humidity over 90% for the past 5 days, and total rainfall for the past 7 days (Madden et al., 1978). Under conditions very favorable for disease development, the number of recommended sprays could equal or even exceed those

applied according to a 7-day schedule. However, in less favorable seasons the number of recommended sprays could be fewer (Pennypacker et al., 1983).

From the FAST model, Madden, Pennypacker, and MacNab derived the forecasting system, *Tomcast*, an acronym for tomato forecast (Pennypacker et al., 1983). Pitblado modified *Tomcast* into the current *Tomcast* model, which relies on leaf wetness and temperature to determine disease severity (Anonymous, 1998a). The *Tomcast* model generates disease severity values (DSVs) as units of disease development for early blight, Septoria leaf spot, and anthracnose. The DSV is a numerical representation of the rate at which disease pressure accumulates on tomato plant tissue. Two factors, leaf wetness and temperature during the leaf wetness hours, determine the DSV. As the number of leaf wetness hours and temperature increases, DSVs increase at a faster rate. Conversely, when there are fewer leaf wetness hours and the temperature is lower, DSVs increase at a slower rate. When the total DSVs exceed a pre-determined limit (spray threshold), a fungicide application is recommended to protect the foliage and fruit from disease. The spray threshold can range from a DSV of 15 to 20 or may extend to a DSV of 35 or 45, depending on grower preference. The DSV a grower should use as a spray threshold usually depends on the amount of disease that can be tolerated based on fruit quality and the intended market for tomatoes.

The objective of this study was to determine the most effective way to use *Tomcast* and azoxystrobin for reducing the use of chlorothalonil in management of leaf diseases in fresh market tomatoes on the Eastern Shore of Virginia.

III-2 Materials and Methods

Experiments for evaluating fungicide treatments in the *Tomcast* system were conducted at the Virginia Tech Eastern Shore Agriculture Research and Extension Center (ESAREC) in Painter, Va. Field trials were conducted in the summers of 1998-2000. Treatments were initiated at varying crop growth stages and predetermined DSV thresholds for fungicide application.

General field preparations for the experiment at the ESAREC included moldboard plowing of a rye cover crop in the early spring. Subsequent field cultivation mixed crop debris into the soil and leveled the soil. Fertilizers were applied according to soil tests and incorporated with a field cultivator, and beds were formed with a soil bed raiser. Trickle irrigation tubing was laid under the plastic mulch that covered the bed at the same time that methyl bromide (45 kg/ha) was applied. Then the beds were covered with plastic mulch and sealed for several days. During this time the irrigation system was assembled. Tomato transplants were planted using a single row transplanter. The rows were staked and plants trellised with three strings as they grew. Plants were watered through high flow trickle irrigation tubing as needed. All experiments used standard fertilizer, herbicide and insecticide recommendations.

The experiments were laid out in a randomized complete block design with four replicates per treatment. Each plot was planted with the variety 'Sunbeam' in rows 9.2 m long, spaced 1.8 m apart, with an in-row plant spacing of 0.5 m. The planting dates were May 7, 1998, May 27, 1999, and May 18, 2000. Treatments were applied at early bloom and repeated on a standard 7-day schedule. *Tomcast* was used to apply treatments after the DSV accumulation reached 25 to 35 units (Madden et al., 1978). Leaf wetness

and temperature values were measured every 30 minutes with a Rainwise Weather-Log Weather Monitoring System (Rainwise Inc., Bar Harbor, ME) at the ESAREC in Painter Va. Weather data values were used to calculate the number of hours of leaf wetness and the average temperature during leaf wetness. This information was used to determine daily DSV units. Treatments were first applied following visual detection of early blight. This occurred during fruit set in 1998, early fruit set in 1999 and full bloom in 2000 (Tables III-2.1-2.2). Treatment rates and schedules are defined in Tables III-2.1-2.5.

Fungicides were applied with a multi-boom sprayer mounted on a high-clearance Hagie™ tractor. The sprayer delivered 373.5 liters/ha at 276 kPa. The spray boom consisted of five nozzles with one nozzle positioned over the row and two nozzles each on two-drop pipes 90 cm from the center nozzle. Distance from the first drop nozzle to the top of the boom was 60 cm and distance from the first drop nozzle to the second drop nozzle was 90 cm. Each nozzle contained a 50-mesh stainless steel strainer and hollow cone TXVS-18 spray tip (TeeJet Spraying Systems Co., Dillsburg, PA).

III-2.1 Ratings

Ratings of foliar early blight disease and Septoria leaf spot disease were taken at various times throughout the duration of the experiments, depending on visual observations of disease development. The percentage of colonized leaves in each plot, based on the Horsfall-Barrett visual rating acuity scale, was recorded twice in 1998 and once in 1999 and 2000. Additional ratings of percentage leaf surface colonized (severity) were taken for early blight in 1998 and 1999 and for Septoria leaf spot in 2000. Percentage defoliation ratings were taken in 1998 and 1999. Mature green fruits were

harvested from six plants per plot, sized and graded for malformation scars, blossom end rot and occasional fruit rots. Harvest dates were July 14 and 30 and August 12 in 1998, August 2, 13 and 27 in 1999, and August 1 and 9 in 2000.

Table III-2.1 Date, temperature in Celsius, relative humidity, cloud cover and the stage of the crop at the time of treatment applications in 1998 and 1999.

1998

Date	*Temperature °C*	*% Relative humidity*	*Wind speed km/hr*	*% Cloud cover*	*Growth stage*
6/3	28.3	53	4.8-8.0	0	Early bloom
6/9	20.0	49	0	100	Full bloom
6/17	25.6	73	4.8-9.6	0	Early fruit set
6/23	27.2	77	8.0-12.8	100	Fruit set
6/25	30.0	62	0-3.2	60	Fruit set
7/1	25.6	57	6.4-9.6	0	Fruit set
7/6	26.1	58	6.4-8.0	0	Green fruit
7/9	21.7	84	0-3.2	100	Green fruit
7/16	27.8	82	6.4-8.0	100	Mature green fruit
7/23	30.0	62	0	0	Early ripe
7/29	29.4	65	3.2-6.4	0	Mature fruit
8/3	23.3	71	14.5-19.3	0	Mature fruit
8/10	28.9	73	3.2-9.6	80	Mature fruit
8/12	28.3	76	4.8-8.0	0	Mature fruit

1999

Date	*Temperature °C*	*% Relative humidity*	*Wind speed km/hr*	*% Cloud cover*	*Growth stage*
6/25	30.6	56	3.2-6.4	60	Pre bloom
7/2	26.7	72	8.0-16.0	0	Bloom
7/9	29.4	68	4.8-8.0	0	Full bloom
7/16	25.6	72	0-3.2	40	Crown fruit
7/20	28.9	69	12.8-16.0	0	Early fruit
7/26	30.0	57	9.6-12.8	40	Mature green fruit
8/4	26.7	55	6.4-11.2	0	Mature green fruit
8/11	28.9	69	8.0-12.8	100	Mature fruit

Table III-2.2 Date, temperature in Celsius, relative humidity, cloud cover and the stage of the crop at the time of treatment applications in 2000.

Date	*Temperature ºC*	*% Relative humidity*	*Wind speed km/hr*	*% Cloud cover*	*Growth stage*
7/3	26.1	63	4.8-8.0	0	Early bloom
7/13	23.3	73	4.8-9.6	0	Full bloom
7/19	28.3	74	3.2-6.4	100	Fruit set
7/28	27.8	68	3.2-8.0	100	Fruit set
7/31	25.0	79	3.2-8.0	100	Mature green fruit
8/8	29.4	79	4.8-8.0	0	Mature green fruit

Table III-2.3 Application timings for fungal disease control in 1998 and 1999.

1998

Timing of application	*Number of*	*Application dates*
7-day treatments beginning at early bloom	10	June 3, 9, 17, 23; July 1, 9, 16, 23,;August 3, 10
1st appl. at fruit set, then 7-day	7	June 23; July 1, 9, 16, 23; August 3, 10
Disease detection, then 7-day	6	July 1, 9, 16, 23; August 3, 10
Tomcast 25 DSV 1st appl., 15 DSV interval	5	June 3, 23; July 6, 29; August 10
Tomcast 35 DSV 1st appl., 15 DSV interval	5	June 17, 25; July 9; August 3, 12
Tomcast 25 DSV 1st appl., 25 DSV interval	3	June 3, 25; July 29
Untreated control	0	-

1999

Timing of application	*Number of*	*Application dates*
7-day treatments beginning at early boom	7	June 25; July 2, 9, 16, 26; August 4, 11
1st appl. at fruit set, then 7-day	5	July 9, 16, 26; August 4, 11
Disease detection, then 7-day	4	July 16, 26; August 4, 11
Tomcast 25 DSV 1st appl., 15 DSV interval	3	July 2, 20; August 4
Tomcast 35 DSV 1st appl., 15 DSV interval	2	July 20 and August 4
Tomcast 25 DSV 1st appl., 25 DSV interval	2	July 29 and August 4
Untreated control	0	-

Table III-2.4 Application of fungicide treatments for fungal disease control in 2000.

Timing of Application	*Number of*	*Application dates*
7-day treatments beginning at early bloom	5	July 3, 13, 19, 31; August 7
1st appl. at fruit set, then 7-day	3	July 19, 31; August 7
Disease detection, then 7-day	4	July 13, 19, 31; August 7
Tomcast 35 DSV 1st appl., 15 DSV interval	3	July 3, 19, 21
Tomcast 35 DSV 1st appl., 35 DSV interval	2	July 20 and 27
Tomcast 45 DSV 1st appl., 15 DSV interval	2	July 19 and 31
Untreated control	0	-

Table III-2.5 Treatment common names and trade names, formulation, and rate for *Tomcast* treatments in 1998-2000.

Common name	*Trade name*	*Formulation*	*Rate a.i./ha*
Chlorothalonil alternated with Azoxystrobin	Bravo WS® Quadris®	6 F 2 SC	1.68 kg 0.1 kg
Azoxystrobin	Quadris®	2 SC	0.1 kg
Chlorothalonil	Bravo WS®	6 F	1.68 kg
[1]Mancozeb+ Copper hydroxide	Dithane® Kocide 2000®	75 DF 66 DF	1.68 kg 1.21 kg
Untreated control	-	-	-

[1] Commercial standard treatment used on Virginia's Eastern Shore

III-3 Results

III-3.1 Control of Early Blight

In 1998, there were no significant ($P \leq 0.05$) differences in early blight incidence or severity among treatments. However, all the treatments proved to be significantly ($P \leq 0.05$) better than the control at reducing defoliation (Table III-3.1). *Tomcast* treatments at 25 DSVs and 25 DSVs resulted in the fewest applications at 70% fewer applications (7 applications) than the standard treatment.

Early blight incidence in 1999 was significantly ($P \leq 0.05$) lower when chlorothalonil and azoxystrobin were applied when 35 DSVs (*Tomcast*) was used to start applications and 15 DSVs was used to determine the timing of subsequent applications (Table III-3.1). The same treatment resulted in 71% fewer applications (4 applications) than the standard treatment with no significant ($P \leq 0.05$) differences in disease severity or defoliation compared to the other treatments in 1999.

The amount of early blight on the Eastern Shore of Virginia was low in 2000 (Table III-3.2). The untreated control had a disease incidence rating of only 8.8; however, three treatments resulted in significantly ($P \leq 0.05$) lower disease incidence than the control. These were all 7-day treatments that included two treatments of chlorothalonil + azoxystrobin, one initiated at fruit set and the other initiated after disease detection. The *Tomcast* treatments, beginning at 25 DSV and continuing at 25 DSV and beginning at 35 DSVs and continuing at 15 DSVs, were not significantly ($P \leq 0.05$) different in disease incidence from the standard treatment applied on a 7-day schedule. Mancozeb + copper hydroxide also provided better disease control than the control. Because the incidence was low, no severity or defoliation ratings were taken in 2000.

None of the treatments were significantly ($P \leq 0.05$) different in marketable yield from the control in the three years the research was conducted (Table III-3.3). The best representation of yield differences was reflected by the percentage of marketable yield and total marketable yield (tons/ha). Tomato yields are difficult to compare because of variations within and between plots.

During each of the three years, the fewest number of applications was made with *Tomcast* schedules with application thresholds of 25 DSVs and 25 DSVs, and 35 DSVs and 15 DSVs.

III-3.2 Control of Septoria Leaf Spot

In 2000, Septoria leaf spot was prevalent and ratings for disease incidence and severity were taken. All treatments provided significantly ($P \leq 0.05$) better disease control than the untreated control, but there were no significant ($P \leq 0.05$) differences among fungicide treatments and application schedules (Table III-3.2). The *Tomcast* treatments beginning with 25 DSVs and continuing at 25 DSVs, and beginning with 35 DSVs and continuing at 15 DSVs resulted in the fewest applications. Septoria leaf spot did not affect the percentage of marketable yield in 2000 (Table III-3.3).

Table III-3.1 Early blight control in staked tomatoes grown on the Eastern Shore of Virginia using *Tomcast* as compared with a weekly fungicide application schedule from 1998 and 1999.

Treatment, application timing and no. of applications[1]	Early Blight					
	1998			1999		
	Inc.[2]	**Sev.**[3]	**Def.**[4]	**Inc.**	**Sev.**	**Def.**
Chlorothalonil/Azoxystobin[5] 7-day (10,7 appl.[1])	43.8 a[6]	18.8 a	21.3 b	15.8 ab	6.0 a	21.3 a
Chlorothalonil/Azoxystrobin 25DSV+15DSV (5,3 appl.)	45.0 a	21.3 a	18.0 b	13.8 ab	5.0 a	30.0 a
Chlorothalonil/Azoxystrobin 25DSV+25DSV (3,2 appl.)	46.3 a	17.5 a	23.8 b	11.8 ab	3.3 a	28.8 a
Chlorothalonil/Azoxystrobin 35DSV+15DSV (5,2 appl.)	55.0 a	25.0 a	26.3 b	10.0 b	2.3 a	21.3 a
Chlorothalonil/Azoxystrobin After detection (6 appl.)	42.5 a	16.3 a	18.0 b	-------	------	--------
Azoxystrobin after detection (6,4 appl.)	47.5 a	23.8 a	18.0 b	13.3 ab	4.5 a	17.5 a
Mancozeb + Copper hydroxide 7-day (10,7 appl.)	38.8 a	16.3 a	15.0 b	11.8 ab	4.3 a	20.0 a
Untreated control (0,0 appl.)	51.3 a	23.8 a	65.0 a	18.3 a	6.3 a	35.0 a

[1] Number of applications (1998 and 1999) for each treatment
[2] Inc. = number of leaves per plant with at least 1 early blight lesion
[3] Sev. = % leaf area affected
[4] Def = % of plant defoliated
[5] Rates of treatments are described in Table III-2.5 and spray intervals are described in Table III-2.3 and 2.4
[6] Values followed by the same letter are not significantly different ($P \leq 0.05$) according to Tukey's studentized range test

Table III-3.2 Foliar disease control in staked tomatoes grown on the Eastern Shore of Virginia using *Tomcast* as compared to a weekly fungicide application schedule in 2000.

	Early Blight	**Septoria leaf spot**	
Treatment, application timing and no. of applications[1]	**Incidence**[2]	**Incidence**[2]	**Severity**[3]
Chlorothalonil/Azoxystobin[1] 7-day (5 appl.)	2.3 b	0.38 b	0.10 b
Chlorothalonil/Azoxystrobin 25DSV+15DSV (3 appl.)	2.8 ab	0.13 b	0.08 b
Chlorothalonil/Azoxystrobin 25DSV+25DSV (2 appl.)	2.5 ab	0.50 b	0.13 b
Chlorothalonil/Azoxystrobin 35DSV+15DSV (2 appl.)	3.0 ab	0.88 b	0.28 b
Chlorothalonil/Azoxystrobin After detection (4 appl.)	2.0 b	1.75 b	0.75 b
Mancozeb + Copper hydroxide 7-day (5 appl.)	2.1 b	4.00 b	2.08 b
Untreated control (0 appl.)	8.8 a	47.50 a	25.50 a

[1] Number of applications (2000) for each treatment. Rates of treatments are described in Table III-2.5 and spray intervals are described in Table III-2.3 and 2.4

[2] Incidence = number of leaves per plant with at least 1 early blight lesion

[3] Severity = % leaf area affected

[4] Values followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Tukey's studentized range test

Table III-3.3 Marketable yield (tons/ha) and the percentage of the yield that is marketable (% marketable fruit is fruit that is blemish-free) in staked tomatoes grown on the Eastern Shore of Virginia following fungicide applications for disease management for 1998 to 2000.

	% Marketable Fruit			Marketable yield tons/ha		
Treatment, application timing and avg. no. of applications[1]	**1998**	**1999**	**2000**	**1998**	**1999**	**2000**
Chlorothalonil/Azoxystrobin 7-day (7.3 appl.[1])	68a[2]	93ab	96a	9.6a	16.9a	22.4a
Chlorothalonil/Azoxystrobin 25DSV+15DSV (3.7 appl.)	67a	90b	95a	9.6a	18.8a	21.8a
Chlorothalonil/Azoxystrobin 25DSV+25DSV (3.3 appl.)	71a	96a	98a	10.0a	19.2a	20.4a
Chlorothalonil/Azoxystrobin 35DSV+15DSV (3.0 appl.)	77a	96a	95a	11.2a	18.9a	17.6a
Chlorothalonil/Azoxystrobin After detection (5.0 appl.)	72a	-	97a	11.2a	-	19.4a
Azoxystrobin after detection (5.0 appl.)	76a	94ab	-	12.5a	17.2a	-
Mancozeb + Copper hydroxide 7-day (7.3 appl.)	74a	93ab	96a	11.5a	18.0a	20.5a
Untreated control (0.0 appl.)	75a	92ab	96a	12.7a	15.4a	21.2a

[1] Average number of applications for each treatment for experiments where the treatment was used. Rates of treatments are described in Table III-2.5 and spray intervals are described in Table III-2.3 and 2.4.

[2] Values followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Tukey's studentized range test.

III-4 Discussion

The Eastern Shore has 780 km of shoreline (Anonymous, 1982). In the estuaries, clams and oysters are produced and natural inhabitants are harvested for seafood. Additionally, there are 261 saltwater farms in Virginia comprising 3,106 ha (Anonymous, 1998b). Run-off from agricultural fields has been suspected to be the cause of high clam larvae mortality. Preliminary data has indicated that run-off from plasticulture fields and water in adjacent creeks contains high concentrations of agricultural chemicals (Dietrich et al., 1996). Also, creek water near selected fields is toxic to sentinel species, such as grass shrimp (Luchenbach et al., 1996).

Chlorothalonil has long been the best control method for fungal diseases in fresh market tomato production on the Eastern Shore (Alexander, 1997). Chlorothalonil is a protectant fungicide that is active only on the plant surface, where it is subject to washing off. Thus, multiple applications are needed to prevent disease. Use of *Tomcast*, a tool to forecast conditions conducive to disease, mandates timely fungicide application. This could potentially reduce the amounts of fungicide applied to a crop of tomatoes. To further reduce pesticide inputs, azoxystrobin can be used as a replacement for chlorothalonil. Azoxystrobin is applied at lower rates and has a low half-life in soil (less than one day) as compared to chlorothalonil, which is moderately persistent. In aerobic soils, the half-life is from 1 to 3 months (Anonymous, 2000). Azoxystrobin is also an acropetal penetrant fungicide, so it is active for longer periods of time, and could improve disease control and allow a greater time interval between applications.

Although use of *Tomcast* did not result in significant improvements on disease control, *Tomcast* did markedly reduce the number of applications (Table III-2.3-2.4,

Tables III-3.1 and III-3.2). Thus, the use of *Tomcast* alone accomplished the objective of reducing the amount of chlorothalonil applied to a tomato crop.

No ($P \leq 0.05$) differences in early blight control were found between treatments in any experiment. However, disease control for all treatments was significantly ($P \leq 0.05$) greater than for the untreated control (Table III-3.1 and 3.2). The results for marketable yield in 1998 and 2000 showed that there were no ($P \leq 0.05$) differences among the treatments. In 1999, there were differences in yield due to disease. The differences were a result of an environmental issue rather than disease control. One treatment incurred high levels of blossom end rot due to irrigation malfunctions.

These results support the use of the *Tomcast* system. The use of *Tomcast* can reduce the number of applications by 40 to 70%, depending on the number of DSV values used to determine the application schedule. The most substantial drop in number of applications per year was in 1998. At that time, a 7-day schedule of chlorothalonil/azoxystrobin resulted in 10 applications, where as the *Tomcast* schedule, based on 25 DSVs followed by a 25 DSV for reapplication, resulted in 3 applications. This represents a reduction of 11.76 kg of chlorothalonil/ha in just the spring crop of one growing season. The reduction in volume of chlorothalonil applied would be tremendous when extrapolated to 1,659 ha of tomaotes.

Azoxystrobin, applied after disease detection in 1998 and 1999, was applied six and four times, respectively. With azoxystrobin applied at 0.1 kg a.i./ha, a total of 0.6 kg/ha active ingredient was applied in 1998 and 0.4 kg a.i./ha was applied in 1999. This represents less pesticide applied in an entire season than is applied in one application of chlorothalonil (1.68 kg a.i./ha).

For resistance management of azoxystrobin, no more than four applications should be made per year according to the label. One or two applications of another fungicide with a different mode of action, such as chlorothalonil or mancozeb, could be used in rotation with azoxystrobin for resistance management. Chlorothalonil or mancozeb are usually applied later in the season when the plants are larger, as is often recommended for resistance management. This would result in more fungicide being deposited onto the plants instead of onto the plastic, further minimizing run-off into surface water.

In summary the use of *Tomcast* and azoxystrobin together can significantly reduce pesticide inputs and costs required for management of early blight and other foliar fungal diseases of fresh market tomatoes grown on the Eastern Shore of Virginia.

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IV. General Discussion

There is no doubt that a reduction in pesticide use would be of benefit to the environment and to the farmer as long as there is not a significant reduction in yield or quality in vegetable production. On the Eastern Shore of Virginia there is a real potential for pesticides to contaminate the sensitive estuaries of the Chesapeake Bay and Atlantic Ocean. Virginia ranks third in fresh market tomato production and essentially all of the commercial tomatoes produced in Virginia are grown on the Eastern Shore. The climate, soils, and access to large markets give the growers of the Eastern Shore a distinct advantage to produce quality fruit and get fair market value for their crop. However, with the production of tomatoes often within meters of estuaries of the Atlantic Ocean and the Chesapeake Bay, producers are faced with potential water quality issues from pesticide run-off.

Plasticulture production systems allow for faster growth of a high quality crop. Plastic mulch protects the plants from contact with soil-borne pathogens while inhibiting the growth of weeds that would require herbicide applications for control. It also helps to increase soil temperatures, which supports a quicker maturation of the crop. The trellis system results in higher yields and high quality fruit by accurately controlling inputs for production. This production system also reduces the infiltration of pesticides into the soil as compared to non-plasticulture production systems. However, the plastic mulch covers about a third of the soil surface, and allows applied pesticides to be washed off and potentially contaminate estuaries where clams are produced.

A report by Deitrich et al., (1996) stated that copper was found in levels toxic to shellfish larvae in waters near tomato production fields. The report also found significant

levels of chlorothalonil in waters near clam beds. This report, along with a lawsuit filed by a clam producer against a tomato grower for losses due to pesticide contamination, led to the need for research to reduce the amount of copper and chlorothalonil used in tomato production.

The main objective of these studies was to reduce the amount of fungicides/bactericides required for production if staked tomatoes. More specifically, the objectives were to reduce the amounts of copper applied for control of foliar bacterial pathogens and chlorothalonil applied for control of foliar fungal pathogens. It would be an understatement to say that the objectives of the studies were met. While the results of the experiments conducted did not provide dramatic improvements in disease control, the levels of the target pesticides were substantially reduced. The data shows that a significant reduction in copper and chlorothalonil can be achieved in tomato production. Using acibenzolar to replace copper on staked tomatoes could reduce copper use on the Eastern Shore by 50% or greater. Acibenzolar was found to be especially effective when copper-resistant or -tolerant bacterial strains were present. It would be a good practice to use a copper pesticide occasionally as a control method for resistance management. The possibilities of resistance development in response to acibenzolar are low; however, alternating chemical control methods would be a good management practice.

A 40% to 70% reduction of total pesticide input for control of fungal diseases of tomato using *Tomcast* was achieved. The use of azoxystrobin in addition to *Tomcast* provided for an even greater reduction in pesticide inputs. Azoxystrobin also represents an alternative fungicide that has better environmental fate characteristics than chlorothalonil.

Future prospects for control of tomato diseases with acibenzolar and SAR-inducing compounds are promising. Virtually every plant has resistance pathways. Chemicals that can induce resistance in other crops should be exploited for disease control. The amount of copper resistance in bacterial populations needs to be evaluated on the Eastern Shore. Best management uses of acibenzolar also need to be determined, as there may questions about which application rates and timing should be used to provide the best and most economical control.

Research using *Tomcast* to extend application intervals should continue to be explored, and growers should be educated about how to implement this tool into their operations. New pesticides and biological control methods for fungal diseases should also be evaluated.

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VITA

ARTHUR S. GRAVES

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EDUCATION

Virginia Polytechnic Institute and State University, Blacksburg, Virginia
Master of Science in Plant Pathology, Expected completion May 2001
Thesis: Replacing copper and chlorothalonil in staked tomato production
Advisor: Dr. Samuel A. Alexander

State University of New York Agriculture and Technology at Cobleskill
Bachelor of Technology in Agronomy, May 1996

RESEARCH EXPERIENCE

Master's Degree Research, *Department of Plant Pathology, Physiology, and Weed Science*
Virginia Tech, Blacksburg, Va. *June 1998 - present*

- Thesis research three summers, 11 trials, bacterial and fungal research in tomatoes.
- Assisted with potato, snap bean, pepper, wheat and pumpkin studies.
- Conducted experimental herbicide efficacy research in imidazolinone-resistant corn cooperatively with doctoral candidate.

Mycogen Seeds, *District Sales Manager, eastern New York and New England* *July 1996 - May 1998*

- Managed plots for corn varieties.
- Organized and conducted plot tours cooperatively with sales representatives.
- Launched Bt and Silage specific hybrids in sales area
- Increased sales $40,000 in first sales season with limited distribution

DowElanco, *Sales Intern, Frankfort, N.Y.* *May - July 1996*

- Aided in the launch of a new herbicide.
- Promoted product line and supported sales through planter calibration
- Assisted dealers with product performance field inspections and grower's concerns

DowElanco, *Summer Research Intern, Waterloo, N.Y.* *May - August 1995*

- Planted, sprayed, harvested and rated multiple insecticide and herbicide research trials and initiated an independent herbicide research study.
- Conducted a product training seminar for sales representatives.

Agway, *Summer Research Intern, Tully, N.Y.* *May - August 1993*

- Assisted with the maintenance of corn, soybean, alfalfa and vegetable research plots.
- Involved in the planting, harvesting, counting populations and rating plots throughout New York, Pennsylvania and Vermont.

TEACHING AND OUTREACH

Teaching Assistant - Pesticide Usage, *Department of Plant Pathology, Physiology, and Weed Science Department*
Virginia Tech, Blacksburg, Va. *January 2001 – May 2001*

- Teaching lab topics of herbicide identification and calibration of pesticide application equipment as well as completing general preparation for labs.
- Designing and grading tests and assignments.
- Advising students during office hours.

Eastern Shore Agricultural Research and Extension Center, *Vegetable Extension Specialist Assistant*
Painter, Va. *May - August 1998 - 2000*

- Presented tomato disease control options to growers and industry representatives during field days.
- Worked with many growers to evaluate problems in the field.
- Created and maintained relationships with growers to work cooperatively in evaluating problems and establishing research plots.
- Operated plant disease clinic for landscape and agricultural problems.

Mycogen, *District Sales Manager, eastern New York and New England* *July 1996 - May 1998*

- Trained sales representatives on new products.
- Organized and hosted field days for growers to provide information on new products and management practices for corn, soybeans and alfalfa.

PRESENTATIONS

Northeast Weed Science Society, Cambridge, Mass., Jan. 3, 2001
Title: Response of imidazolinone-resistant corn to CGA-362622

Department of Plant Pathology, Physiology, and Weed Science Seminar, Virginia Tech, Blacksburg, Va., Oct. 25, 2000
Title: Replacing copper and chlorothalonil in staked tomato production on Virginia's Eastern Shore

Potomac Division, American Phytopathological Society, Newark, Del., March 23, 2000
Title: Alternatives to copper for bacterial disease control in staked tomatoes

PUBLICATIONS

Graves, A.S., Alexander, S.A., Waldenmaier, C. Reducing copper usage in environmentally-sensitive areas for control of tomato bacterial diseases, Plant Disease, Journal publication. (being prepared)

ABSTRACTS

Graves, A.S., Richardson, R.J., Wilson, H.P., Heins, T.E. 2001. Response of imidazolinone-resistant corn to CGA-362622. Northeast Weed Science Society, Cambridge, Mass.

Graves, A.S., Alexander, S.A. 2000. Alternatives to copper for bacterial disease control in staked tomatoes. Potomac Division, American Phytopathological Society, Newark, Del.

SPECIAL TRAINING AND LICENSES

Virginia Pesticide Applicator License - present
Certified Crop Advisor - March 1998 - December 2000
New York Pesticide Applicator License - 1995-1998

HONORS / AFFILIATIONS

Graduate Student Organization, treasurer, Department of Plant Pathology, Physiology, and Weed Science 2000-2001
Virginia Tech Weed Science Team Member 1999 (third place individual NEWSS Contest)
American Phytopathology Society Member
Northeast Weed Science Society Member (NEWSS)
SUNY Cobleskill Agronomy Student of The Year 1995
SUNY Cobleskill Weed Science Team Member (first place 1996, second place 1995 - NEWSS Contests)

ARTHUR S GRAVES II