RESEEDING TOMATO PRODUCTION IN WEST AFRICA: IDENTIFICATION AND DEPLOYMENT OF HIGH YIELDING CULTIVARS RESISTANT TO TOMATO YELLOW LEAF CURL DISEASE

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RESEEDING TOMATO PRODUCTION IN WEST AFRICA: IDENTIFICATION AND DEPLOYMENT OF HIGH YIELDING CULTIVARS RESISTANT TO TOMATO YELLOW LEAF CURL DISEASE

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First identified in the Middle East in the 1930s, Tomato Yellow Leaf Curl Disease (TYLCD) is a whitefly-vectored viral disease of tomato that has spread rapidly throughout the world in the last two decades and is now one of the most serious constraints to tomato production worldwide. Significant efforts have been invested in its control, resulting in the development of numerous techniques and resistant cultivars that have minimized the disease's impact wherever they are deployed. However, lack of access to agricultural inputs in developing regions such as West Africa has delayed the adoption of effective TYLCD control methods, leading to stagnant or volatile tomato yields. As demand for tomatoes has increased among West Africa's growing urban population, imports of canned tomatoes have skyrocketed, demonstrating the potential for a viable West African tomato processing industry but highlighting the need for the higher and more consistent yields required for a cannery to be profitable.

This dissertation describes a project in which a multinational, highly collaborative germplasm trialing network has been established in West Africa to identify vegetable cultivars well-adapted to the region and to mobilize those varieties into local seed distribution networks. Research partners in seven countries have evaluated 70 cultivars from both public and commercial breeding programs over the course of three years,

focusing on TYLCD resistance, yield, and fruit quality. Currently, efforts are underway to distribute seeds of the most successful cultivars to farmers in West Africa.

BIOGRAPHICAL SKETCH

The author was born on May 16, 1980. From 1998 to 2002 he studied at Yale University in New Haven, Connecticut, where he received a B.S. degree in Molecular, Cellular and Developmental Biology with a concentration in neurobiology. He then moved to Ithaca, New York to work as a research assistant at the Boyce Thompson Institute for Plant Research. During that time he studied the molecular genetics of a bacterial pathogen of tomato. In 2004 he enrolled in a graduate program at Cornell University to study diseases of tomato in the developing world.

for Sara

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CHAPTER 1

TOMATO YELLOW LEAF CURL VIRUS AND ITS CONTROL

I. *The emergence of TYLCV as a global threat to tomato production*

In the summer of 1939 the farmers of the Jordan Valley, in what is now the State of Israel, observed a new disease on tomato (*Solanum lycopersicum*) of unknown etiology (Avidov, 1946). Within a month of transplanting, their tomato plants showed a highly unusual growth pattern characterized by severe stunting, erect shoots, and small, misshapen leaflets. Most strikingly, leaves developing after infection were highly chlorotic and showed an upward curling at the leaflet margins. Plants infected while still young were found to produce almost no marketable fruit at maturity. Given the symptoms, the disease was named Tomato Yellow Leaf Curl Disease (TYLCD, Cohen and Harpaz, 1964).

TYLCD remained an occasional problem in the Jordan Valley for two decades until the summer of 1959, when a devastating outbreak of the disease decimated all tomatoes in the region and reduced yields to zero for the season (Cohen et al., 1961). Concerned that the disease had rapidly become significantly more problematic, farmers sought help from the Israeli Ministry of Agriculture. The Israeli Agricultural Research Organization (ARO) stepped in and determined that the disease outbreak was strongly correlated with a significant increase in whitefly (*Bemisia tabaci*) populations. The late 1950s had seen the initiation of large-scale cotton cultivation for commercial production in the Jordan and Bet She'an Valleys, and since cotton was a preferred host of the local whitefly biotype, the insect's population had skyrocketed (Cohen and Lapidot, 2007). Controlled transmission tests in the laboratory soon confirmed that the disease was whitefly-vectored and viral in nature (Cohen and

Harpaz, 1964). As a result the causal agent was named *Tomato yellow leaf curl virus* (TYLCV).

In subsequent years, TYLCD-like diseases began to be described throughout the Mediterannean basin and the Greater Middle East. Very similar disease symptoms were observed in Sudan in 1965 (Yassin and Nour, 1965), Egypt in 1966 (Makkouk and Laterrot, 1983), Tunisia in 1967 (Cherif and Russo, 1983), Saudi Arabia in 1971 (Mazyad et al., 1979), Cyprus in 1974 (Ioannou, 1985), Jordan (Abu-Gharbieh et al., 1978) and Lebanon (Makkouk et al., 1979) in 1976, and Iraq in 1978 (Makkouk, 1978). The mid-1970s also saw the emergence of similar virus symptoms on tomato in West Africa, being described in Nigeria (Lana and Wilson, 1976), Senegal, Cape Verde, The Gambia, Mauritania, Côte d'Ivoire, and Mali (D'hondt and Russo, 1985) in the late 1970s and early 1980s.

Notably, similar whitefly-transmitted tomato viruses were described in the Indian subcontinent as early as 1948 (Vasudeva and Raj, 1948), and in Central and South America in the 1970s (reviewed by Polston and Anderson, 1997; and Varma and Malathi, 2003). It was clear from early on that these diseases were somewhat different in their symptomology from the "classic" TYLCDs, but it was not until the development of molecular tools for the more detailed characterization of these viruses in the late 1980s (Czosnek et al., 1988; Navot et al., 1989) that they were identified as different clades of virus species having diverged from the Mediterranean and African viral species as long as 130 million years ago, in the case of the New World isolates, with the separation of the Americas from the Gondwana landmass (Seal et al., 2006).

The early 1990s saw an explosion in the prevalence and importance of Mediterranean TYLCD throughout the world. The disease caused significant losses of tomato crops in the Western Mediterranean, starting in Sicily in 1989 (Credi et al., 1989) and spreading by the early 1990s throughout Italy and Spain (Moriones et al., 1993), arriving in Portugal in 1996 (Louro et al., 1996). Meanwhile, eastern Mediterranean TYLCV was inadvertently introduced in the Dominican Republic in 1994 (Nakhla et al., 1994), and quickly spread throughout the Caribbean (Bird et al., 2001; McGlashan et al., 1994; Ramos et al., 1996) and into the southeastern United States (Polston et al., 1999; Valverde et al., 2001). In 1998 the same eastern Mediterranean virus was also introduced in Japan (Kato et al., 1998), and by 2006 had been identified in Australia (Tesoriero and Azzopardi, 2006), China (Wu et al., 2006), and Mexico (Brown and Idris, 2006). It was identified for the first time in California, the largest tomato-producing region of the world, in 2007 (Rojas et al., 2007).

Recent phylogenetic studies have determined that the TYLCD-associated viruses from the Mediterranean Basin and northern Sub-Saharan Africa consist of six distinct virus species, commonly known as the TYLCV cluster (Abhary et al., 2007). The Eastern Mediterranean species first isolated in Israel is known as *Tomato yellow leaf curl virus* (TYLCV), and is the species that has become truly worldwide in distribution in the last decade. The Western Mediterranean species common throughout Italy and Spain is known as *Tomato yellow leaf curl Sardinia virus* (TYLCSV), though importantly TYLCV has also been a serious problem in that region since its introduction through Portugal into Spain in the mid-1990s. Recombination between TYLCV and TYLCSV has created two very new virus species, *Tomato yellow leaf curl Malaga virus* (TYLCMalV, discussed in detail below, Monci et al., 2002), and *Tomato yellow leaf curl Axarquia virus* (TYLCAxV, Garcia-Andres et al., 2006), both likely emergent

only within the last decade. TYLCSV has also been found in Jordan, Morocco, and Tunisia. The TYLCD-associated virus species most prevalent in Sub-Saharan Africa is known as *Tomato yellow leaf curl Mali Virus* (TYLCMLV), and has been found throughout West Africa in Mali, (Dembele and Noussourou, 1991), Ghana (Osei et al., 2008), Benin, Burkina Faso, Niger, Senegal, and Togo (Chen et al., 2009) as well as in Ethiopia (Shih et al., 2006). The sixth species in the TYLCV cluster is *Tomato yellow leaf curl Sudan virus* (TYLCSDV), which has been found only in Sudan and Yemen (Abhary et al., 2007). There are an additional 51 known species of whitefly-transmitted tomato viruses outside of the TYLCV cluster (Fauquet and Stanley, 2005). These species have localized distributions throughout the world, particularly in the Americas and in Southern Asia. While their international spread has been minimal, their local importance in causing TYLCD-like diseases, sometimes in conjunction with a TYLCV-cluster virus and sometimes not, is undeniable and they are often highly adapted to their environments and highly destructive of tomato crops. In fact, it has been argued that the TYLCV cluster has not appeared in South America and in India because it cannot compete with the locally-adapted whitefly-transmitted tomato viruses (Abhary et al., 2007).

There are numerous factors, both genetic and environmental, that set the stage for the rapid worldwide proliferation of TYLCD in the last two decades. This section addresses the particular features of the causal viruses, their vector, their host and their environment that have caused TYLCD to become the dominant constraint to tomato production worldwide in recent years.

Virus Nomenclature

Given the complexity of the family of whitefly-transmitted tomato-infecting viruses, nomenclature rules have been laid out to ensure consistent naming practices (Fauquet and Stanley, 2005). Viruses are grouped into species, strains, and isolates, while the word variant is reserved for describing different viruses outside the context of proper nomenclature. Species are defined as having greater than 89% sequence similarity between all members. Within a species, variants showing different levels of infectivity or different symptomologies are known as distinct strains. Each individual instance of a virus that is cloned and sequenced is known as an isolate. Virus species names can be abbreviated (e.g. TYLCV) and strain names are separated from the species name by a hyphen. Thus, TYLCV-IL is the original TYLCV strain from Israel, and TYLCV-Mld is a strain causing more mild symptoms. Within the 6 species of the TYLCV cluster there are a total of 15 strains. When relevant, isolates are differentiated by square brackets. Thus, TYLCV-IL[IT] is a TYLCV-IL isolate from Italy, while TYLCV-IL[DO] is a TYLCV-IL isolate from the Dominican Republic. More specific information, such as collection city or year, can be specified within the isolate designation if necessary to differentiate between isolates from the same country. In this work, TYLCV will be used as the generic term for all six species in the TYLCV cluster. When greater specificity is necessary, strain indicators will be provided.

TYLCV Biology

All species causing TYLCD-like diseases in tomato are members of the family Geminiviridae and the genus *Begomovirus* (Fauquet and Stanley, 2005). Members of the family *Geminiviridae* (known as geminiviruses) are identified as having single-stranded DNA genomes consisting of one or two ~2.8 kb circular DNA molecules encapsidated in unique twinned, or geminate (hence the name geminivirus), quasi-

isometric virion particles of ~20-30 nm in size (Jeske, 2009). There are four genera within the family *Geminiviridae*, distinguished by their host range, insect vector and genetic organization (Rojas et al., 2005). Genus *Mastrevirus* (type member: *Maize streak virus*, MSV) consists of viruses with monopartite genomes that are vectored by leafhoppers and typically infect monocotyledonous plant species. Members of the genus *Curtovirus* (type member: *Beet curly top virus*, BCTV) also have monopartite genomes, but with a different genome organization, and are also vectored by leafhoppers, albeit different leafhopper species. Their hosts are typically dicotyledonous plants, and though there are few distinct curtovirus species, each is capable of infecting many host plant species. Genus *Topocuvirus* has only one member, *Tomato pseudo-curly top virus*, which has a similar genome organization to that of curtoviruses, but is vectored by a treehopper species. Finally, genus *Begomovirus* (type member: *Bean golden mosaic virus*, BGMV) is by far the largest of the four geminivirus genera, containing the vast majority of known geminiviruses (132 species as detailed in Fauquet and Stanley, 2005). Most begomoviruses have bipartite genomes, though many of the Old World tomato-infecting begomoviruses (including all members of the TYLCV cluster) have monopartite genomes. Begomoviruses are vectored by the whitefly *Bemisia tabaci* (Gennadius), an agricultural pest with worldwide distribution and an exceedingly broad host range. In contrast to their whitefly vectors, most begomoviruses have a very narrow host range, infecting just a small set of related dicotyledonous plant species.

All six species in the TYLCV cluster have monopartite genomes of ~2.75 kb with just six overlapping open reading frames (ORFs), two in the sense orientation of the viral genomic strand (known as V1 and V2) and four in the complementary strand (known as C1 through C4) (Moriones and Navas-Castillo, 2000). Despite its miniscule genetic

arsenal, TYLCV is highly adapted to its host and vector and is capable of executing a wide range of surprisingly far-reaching functions to catalyze its replication and distribution. These include entry into the host phloem via whitefly feeding, entry into host cells, movement of viral DNA into the nuclei of infected host cells, synthesis of a complementary genomic strand to make double-stranded DNA, synthesis of viral proteins, replication of viral genomic DNA, movement of viral genome molecules out of the nucleus and into neighboring cells, encapsidation of viral genomic molecules in a protein capsid capable of protecting the DNA during transit in the whitefly vector, and movement of virion particles into the plant phloem to allow for long-distance movement within the plant or acquisition by a feeding whitefly. In the process, the virus evades host defense systems, most notably by suppressing the plant's post transcriptional gene silencing (PTGS) system, and alters the host metabolism both to favor the synthesis of viral particles and to generate symptoms, such as yellowing, that attract whiteflies to feed (Hanley-Bowdoin et al., 1999; Jeske, 2009; Rojas et al., 2005).

Among this long list of activities are some that are significantly more complex and involved than might immediately be apparent. For instance, because the virus lacks its own DNA polymerase complex, it must recruit the DNA-replication machinery of host cells to copy its genome. However, the vast majority of plant cells are terminally differentiated and lack DNA-replication enzymes. Thus, one of TYLCV's six genes has been shown to interfere with the host plant's cell cycle, causing the infected cells to reenter S-phase and reinitiate DNA replication and, in the process, replicate the virus's own genome. This viral gene has strong sequence identity to a human cell cycle regulator known as retinoblastoma protein or pRb, a mutation of which is implicated in the unchecked cell growth of certain retinal tumors (Hanley-Bowdoin et

al., 1999). The virus additionally encounters constraints that inhibit its movement from cell to cell in the plant: while most plant cells are connected by pores known as plasmodesmata, those pores are typically too small, and too well-regulated, to allow a large DNA molecule like a begomovirus genome to pass through. Nonetheless, studies have shown that during TYLCV infection plasmodesmata are modified by the virus so that ssDNA molecules as large as 2.8 kb, whether associated with the virus or not, are capable of passing from cell to cell (Gilbertson et al., 2003). This upper limit of 2.8 kb appears to be the driving force keeping the geminivirus genome small – larger genome molecules cannot spread cell-to-cell during infection, and thus geminiviruses are severely constrained in their coding capacity. In some instances, TYLCV has evaded this limitation by adopting a satellite DNA molecule known as a DNA-β, which will be discussed later.

Molecular Factors Affecting the Evolution of TYLCV

There are features of TYLCV genetics that have allowed the virus to evolve quickly and thus to adapt to new environments and new hosts, leading to the worldwide expansion of the disease over the last two decades. The first such feature is a high mutation rate, and the second is a high rate of recombination between related TYLCV variants.

High mutation rate

DNA repair is a critical molecular function in most species that serves to prevent frequent mutations during regular DNA maintenance activities. Proofreading enzymes scan for mismatched base pairings in double-stranded DNA deriving from polymerase errors or oxidative damage and correct the mismatches before they can be propagated. RNA viruses typically use their own error-prone polymerases during genome

replication, and thus have a high mutation rate, but since DNA viruses typically use host DNA replication machinery one might expect them to have much lower mutation rates. A recent study of TYLCV sequences collected from around the world during the last two decades, however, shows that despite being replicated in nuclei with significant proofreading capabilities, TYLCV has a nucleotide substitution rate comparable to those of RNA viruses – approximately 3×10^{-4} substitutions/site/year in coding regions, and approximately 1.5×10^{-3} substitutions/site/year in noncoding regions (Duffy and Holmes, 2008). While it is unclear exactly what mechanism is responsible for the elevated mutation rate, there are several possibilities. Most species typically use methylation to differentiate between the template strand and the new strand in replicating DNA, allowing mismatches to be corrected in favor of the template. However, it is possible that geminivirus DNA molecules do not carry the proper methylation pattern, and therefore either cannot be corrected accurately or have a tendency to be corrected in favor of the error. Alternatively, the mechanisms of geminivirus DNA replication might prevent error correction even if the correct methylation pattern were present. Geminivirus genomes often replicate by rolling circle replication (RCR, Hanley-Bowdoin et al., 1999), in which the DNA is only transiently double stranded and therefore may not be accessible to proofreading enzymes. Geminiviruses might also recruit a more error-prone DNA polymerase (perhaps one lacking a base-excision repair mechanism altogether) from the host's nucleus for their own genome replication. However, the patterns of nucleotide substitutions observed within the TYLCV cluster suggest an alternative explanation. The TYLCV sequence data show a marked bias towards C→T and G→A transitions at the expense of all other nucleotide substitutions. These two substitutions are both commonly attributed to the deamination of bases, in which cytosine is converted to uracil (which base pairs with adenine) and guanine is converted to xanthine (which

base pairs with thymine). Deaminating enzymes, which typically attack ssDNA, have been identified in plants, implying that the high mutation rate observed in TYLCV might be attributable to the deamination of the ssDNA genome rather than an avoidance of proofreading functionality during replication (Duffy and Holmes, 2008).

Recombination

While nucleotide substitutions are the ultimate source of genetic variation within the TYLCV cluster, recombination plays a very significant role in creating new genetic combinations that allow TYLCV to adapt to new environmental conditions and ecological niches. Homologous recombination is a regular part of the begomovirus infection cycle, and the vast majority of the viruses causing TYLCD-like diseases have been determined to be ancient recombinants (Moriones et al., 2007; Seal et al., 2006). Though the precise mechanisms of recombination in geminiviruses are not fully understood, recent work has shown that, in addition to using RCR for DNA replication, geminiviruses also use a mechanism known as recombination dependent replication (RDR) in which the replication of genomic DNA is coupled with a recombination event (Preiss and Jeske, 2003). This mechanism appears to be widespread throughout the geminiviruses. When two or more viruses infect the same plant cell, RDR can lead to new hybrid variants that, if well-adapted in the population, may become widespread and even outcompete their parents.

Given the distribution and evolutionary history of tomato-infecting begomoviruses, it seems highly unlikely that tomato has always been (or is even currently, in some cases) their primary host. Tomato, like all other crop species, was only domesticated within the last 10,000 years, and was not introduced to the Old World until the 16th century. It stands to reason that many of the tomato-infecting begomoviruses

identified throughout the world, which have been evolving in their respective environments for many millions of years, are also capable of infecting other plant species. In fact, this is very much the case. Most plant viruses are capable of infecting a range of wild species, often known as reservoir hosts since they maintain a pool of viral inoculum even when all individuals of the cultivated host are removed. Tomato-infecting begomoviruses are no exception – most infect a range of reservoir species that are indigenous to their native region (Bedford et al., 1998; Cohen et al., 1988; Salati et al., 2002).

When a highly virulent and infectious TYLCV variant is introduced to a new region (typically through human activity), it may not be well adapted to the locally preferred tomato varieties, the native reservoir host species, or the particular whitefly population in its new environment. However, co-infection of a plant with a locally-adapted begomovirus offers the opportunity for recombination and the generation of a hybrid virus possessing both the local adaptations of the native begomovirus and the virulence and infectivity of TYLCV. The result is often a highly infectious TYLCV strain that results in an epidemic. Recombination with local begomoviruses also allows TYLCV to overcome the genetic bottleneck associated with the founder effect – while the introduced variant might carry little genetic diversity that would allow it to adapt to its new environment, that diversity is often available in the local begomovirus population and thus TYLCV is readily capable of adapting to most tomato-growing regions of the world (Roossinck, 1997; Seal et al., 2006).

There are numerous examples in the literature that illustrate the potential of recombination to drive TYLCV evolution and its colonization of new environments (reviewed by Moriones et al., 2007). Recent surveys in Italy and Spain have revealed

infections by several different tomato-infecting begomoviruses in the same field, and even in the same tomato plant, during outbreaks of TYLCD (Moriones and Navas-Castillo, 2008). In addition, a survey of begomoviruses in southern Spain in the weed *Solanum nigrum* (European Black Nightshade), a known reservoir host of TYLCV, revealed infections by multiple TYLCV species in a single plant (Bedford et al., 1998). Both of these observations demonstrate that opportunities for recombination between different tomato-infecting begomoviruses are frequently available. A laboratory study of recombination in tomato between TYLCV-IL and TYLCSD showed that recombination can occur quickly, with a recombinant form accounting for as much as 50% of the virus progeny isolated within just one infection cycle (Moriones et al., 2007). Notably, TYLCV-IL and TYLCSV co-infections are commonly found in nature (Garcia-Andres et al., 2006; Monci et al., 2002).

Phylogenetic studies of extant members of the TYLCV cluster have shown all of these viruses to be patchworks of recombined elements, with sequence matches between any two viruses typically spanning only a portion of each virus's genome (Abhary et al., 2007). For instance, TYLCV and TYLCSV are closely related species, but sequence analysis shows that they differ significantly from each other, and from the other members of the TYLCV cluster, in a small region containing portions of ORFs C1 and C4 and the intergenic region. In that region TYLCV-IL has greatest sequence similarity to an Indian tomato-infecting begomovirus species named as *Tomato leaf curl Karnataka virus*, and the closest match for TYLCSV in that genomic region is *South African cassava mosaic virus* (Moriones et al., 2007). This implies that the common ancestor of TYLCV-IL and TYLCSV underwent separate recombination events with an Asian begomovirus and an African begomovirus to yield the current strains associated with TYLCD. It is plausible that these recombination events gave

the ancestors of TYLCV-IL and TYLCSV a greater host range or some other selective advantage in their respective environments that allowed them to become the highly adapted viruses they are today.

Since the early 1990s, researchers in Spain have been carefully monitoring tomato-infecting begomovirus populations to ensure a quick reaction to any new introductions. As a result, they have been able to observe recombination in action as TYLCV adapted to the ecological niche in Spain. The initial colonization of Spain by TYLCV took place in the early 1990s with the introduction of TYLCSV-ES (Noris et al., 1994). Given the limited genetic variation in the introduced viral population the virus remained stable but with relatively low infectivity and virulence. However, the introduction of TYLCV-IL and TYLCV-Mld was reported in 1997 (Navas-Castillo et al., 1997), providing greater genetic variation and opportunities for recombination with the locally established strain. Sure enough, in 2002 a report was published describing a novel species named *Tomato yellow leaf curl Málaga virus* (TYLCMalV), a recombinant between TYLCSV-ES and the TYLCV-Mld (Monci et al., 2002). This naturally-occurring variant soon proved to be more highly adapted than either of its two parents and quickly became the dominant tomato-infecting begomovirus in southern Spain, providing a real-world contemporary illustration of the power of recombination to yield new, highly-adapted begomoviruses.

DNA-β satellites

First discovered in 1997 (Dry et al., 1997), DNA-β satellites are DNA molecules of approximately 1,360 nucleotides that have been found to be associated with some begomoviruses (reviewed in both Briddon et al., 2008; Mansoor et al., 2003). Incapable of independent replication, DNA-βs depend on their associated helper

begomovirus for their accumulation, movement and encapsidation. However, many helper viruses are incapable of inducing disease symptoms without the help of a DNA-β satellite. DNA-β molecules consist of a degenerate replication-initiation site that apparently allows a given satellite to be replicated by a range of different begomoviruses, increasing opportunities for transfer from one helper virus to another during mixed infections. In addition, DNA-βs carry a single open reading frame known as βC1, which has been described as a pathogenicity determinant and a suppressor of post-transcriptional gene silencing, and has been shown to increase levels of viral DNA *in planta* and to bind to viral DNA and potentially be involved in viral movement. DNA-βs have been associated with some of the worst outbreaks of begomovirus-associated diseases in recent years, and given their significant diversity and their ability to switch associations between helper viruses it is likely that they are a major factor in both the adaptation of begomoviruses to new niches and in the outbreaks of new begomovirus-associated diseases.

The Whitefly Vector – Bemisia tabaci

While begomovirus biology is an exceedingly important player in the evolution of the complex of viruses causing TYLCD throughout the world, it is not the only factor involved. An equally important factor in the transmission of TYLCV from one plant to the next is its insect vector, the whitefly *Bemisia tabaci*.

Bemisia tabaci, sometimes known as sweet potato whitefly, is a hemipteran insect native to arid tropical and subtropical regions throughout the world (reviewed in Brown, 2007). It has an exceedingly broad worldwide distribution and can overwinter in any location without sustained periods of freezing temperatures – in colder climates, it is not uncommon to find whiteflies adapted to protected greenhouse conditions. Like

all hemiptera, *Bemisia tabaci* feeds by piercing plants with its specialized mouthparts and sucking the sap from the phloem. There is a significant amount of diversity within the *Bemisia tabaci* species, and numerous biotypes exist with different feeding patterns – some biotypes are highly polyphagous, feeding on a wide range of plant species, while others have narrow host ranges and are occasionally even limited to a single plant species. Reproduction in *Bemisia* whiteflies follows an unusual arrhenotokous parthenogenetic pattern, in which unmated females lay haploid eggs destined to become males, and mated females can lay haploid male eggs or diploid female eggs. The eggs are laid while the female is feeding on a plant, and are deposited in a semi-circular pattern on the underside of the leaf as the female swivels around the feeding site. *Bemisia* whiteflies are poor fliers, but regularly travel 10 km in the wind (Cohen et al., 1988), and around the globe in association with the transport of plants by humans (Caciagli, 2007).

Virus-Vector Interaction

The relationship between begomoviruses and *Bemisia tabaci* is an exclusive one – no other insect can vector the viruses. This implies significant molecular specificity between the virus and its vector, but while the path of the virus through the vector has been described (Brown, 2007), little is known about the molecular interactions. Upon being taken up by the whitefly, the begomovirus travels with the plant sap to the insect's gut, where it crosses the gut barrier and enters the hemolymph. The virus circulates in the hemolymph and enters the salivary gland, where it waits until it can be released into saliva and thus enter a new host plant. In this process the only known molecular interaction takes place between the coat protein of the virus and a heat shock protein (HSP60) synthesized by the endosymbiont *Candidatus portiera aleyrodidarum*, a bacterium that lives in the whitefly gut and synthesizes some amino

acids not found in sufficient quantities in plant sap. The HSP60 is believed to act as a chaperone for the viral capsid, helping it to maintain its structural integrity as it makes its way from the gut to the salivary gland. While no other molecular interactions have yet been described, it is believed that the viral capsid does interact with various whitefly receptors as it makes its way from the gut to the salivary gland, and also with other factors within the whitefly that stabilize the capsid and possibly protect the whitefly from developing an immune response to the virion particle. Genetic evidence points to an association between mutations in the begomovirus coat protein and particular genotypes of the whitefly vector (Seal et al., 2006). These associations imply coevolution of the virus and the vector, and support the existence of molecular interactions between the viral coat protein and proteins in the whitefly.

The B-Biotype

The explosion of TYLCV starting in the late 1980s is strongly correlated with the emergence of a new whitefly variant, the B biotype, that first began appearing globally in the early 1980s (Brown, 2007; Polston and Anderson, 1997; Schuster et al., 1990). The B biotype is a relatively new whitefly biotype of unknown origins with several features making it a particularly effective begomovirus vector. It is highly polyphagous, feeding on a wide variety of fiber and vegetable crops including bean, cotton, cucurbits, eggplant, pepper, okra, tomato, brassicas, *Lantana*, soybean, sesame, and a number of ornamental species. This makes the whitefly well-adapted to a wide range of environments, and increases opportunities for begomoviruses to spread to new hosts, potentially increasing their host range and offering greater opportunities for recombination and the acquisition of new DNA-β satellites. In addition, the *Bemisia tabaci* B biotype has very high fecundity, with a single female producing as many as 300 eggs, almost 5 times the number of many other biotypes (Bedford et al., 1994;

Brown, 2007). The B biotype is also resistant to certain pesticides, such as carbamate and organophosphate insecticides, that are often used to control outbreaks of whiteflies (Brown, 2007). Finally, the B biotype causes phytotoxicity in its own right, causing irregular ripening in tomatoes (Schuster et al., 1990) and generating a symptom known as "silverleaf" in a number of other crops.

The accidental introduction of the B biotype of *Bemisia tabaci* to the USA and Caribbean from an unknown location in 1986 first brought this whitefly variant to the attention of the agricultural community and raised awareness of the potential for whiteflies to be invasive vectors of disease (Polston and Anderson, 1997; Schuster et al., 1990). As the whitefly spread, new begomoviruses began to be reported throughout the tropical Americas. In 1991 B biotype populations exploded, reaching unprecedented levels in irrigated cropping systems in the Southwestern USA, the Caribbean, and the tropical Americas, reaching South America in 1994. In the following years outbreaks were reported in Australia, China, Egypt, Mediterranean Europe, Israel, Japan, Pakistan and Turkey. In many regions, the B biotype has replaced endemic biotypes. However, one other biotype, known as the Q biotype, has shown to be competitive with the B biotype and is recognized as a rising threat (Brown, 2007). The Q biotype, which appears to have originated in southern Spain, is highly polyphagous and has high fecundity, similar to the B biotype. However, it is resistant to a different group of pesticides, so that pesticides currently used to control the B biotype are unlikely to be effective on the Q biotype. As of 2005 the Q biotype was identified on ornamentals in China, Japan, Mexico and the USA. It is expected that without proper controls on global plant movements, the Q biotype will pose a major threat to agricultural production in the coming years (Brown, 2007).

Human Activities

While begomovirus evolution and the emergence of new whitefly biotypes are strongly associated with the explosion of TYLCD throughout the world's tomato-growing regions in the last two decades, there is no doubt that human activities have been an integral factor in shaping the development of this plant disease pandemic. Two human activities in particular can be implicated in the increasing severity of TYLCD around the globe – agricultural intensification, and the global transport of plant materials.

Agricultural Intensification

The last century has seen a gradual but remarkable change in the way agriculture is conducted around the globe. While these changes are highly multifaceted, they tend to include a trend towards larger farms growing a less diverse collection of crop varieties in environments that are made more homogenous through soil amendments and irrigation (Matson et al., 1997). These changes have drastically increased crop yields and consequently decreased the overall cost of food production, but have done so at the expense of the diversity of the ecology of the farming environment, thereby altering the nature of the evolution of pests and diseases. In heterogeneous environments in which multiple crop varieties are grown, a disease that evolves to exploit a weakness of a particular crop variety is limited in host range to only that crop variety or any others that share the same weakness. In a monocropping system, however, all plants in the same field, across the same region, and often even across the globe share the same genetics and therefore the same weakness, vastly increasing the evolutionary fitness of that newly emergent disease variant. Increased fitness leads to an increase in the replication of the disease, which in turn leads to an increase in opportunities for mutations or recombination events that further increase fitness

(Thresh, 1982). Thus agroecological homogeneity tends to rapidly increase the rate of development of new disease variants with higher virulence and infectivity.

It is precisely these features of agricultural homogeneity that have led to the explosion of the TYLCV cluster of viruses and the B biotype of the *Bemisia* whitefly (Varma and Malathi, 2003). The cultivated tomato species *Solanum lycopersicum* has a very narrow genetic base, containing less than 5% of the variation contained within its wild relatives (Bai and Lindhout, 2007), and high yielding processing cultivars with even less genetic diversity have become the norm in many parts of the world in the last several decades. Tomato has also increased in global popularity during that time, leading to a significant increase in the land area under tomato cultivation (FAOSTAT, 2009). TYLCV has precisely evolved to exploit commonly shared genetic weaknesses in all tomato cultivars grown across the world. In the meantime, the non-genetic features of environmental homogeneity in agroecosystems have been exploited by whiteflies. As the mass-marketing of agricultural chemicals has become a global enterprise, *Bemisia* whiteflies have evolved resistance to the most popular pesticides (Brown, 2007). Whiteflies have also adapted to exploit modern efficient irrigation systems. Many tomatoes are grown in arid areas that are too dry for most insects, and are irrigated by means of efficient drip lines that deliver water directly to the plant roots. However, whiteflies exclusively obtain water from plant phloem, and therefore the increased prevalence of irrigation has actually increased the geographic distribution of whiteflies (Seal et al., 2006).

Global Movement of Plant Materials

While agricultural intensification has created optimum environments around the world for the development of new, more debilitating plant diseases, the spread of those

diseases would be severely limited without the global movement of plant materials. Whiteflies are capable of regional movement – if a single whitefly can move 10 km in a week by wind dispersal (Cohen et al., 1988), it stands to reason that over the course of several years viruliferous whiteflies could spread significant distances over land, provided the proper wind currents and the existence of virus-susceptible vegetation every 10 km or so. However, given their poor flying abilities and significant fragility, it seems highly unlikely that whiteflies could cross oceans (Byrne and Bellows, 1991), and evidence strongly supports the notion that the TYLCV outbreaks in the Caribbean and the Americas, Australia, Japan, Cape Verde, and Réunion were all caused by the introduction of the virus from the Mediterranean Basin (Abhary et al., 2007). As a result, it is recognized that the international movements of plant materials by humans is largely responsible for the global spread of TYLCV in the last two decades.

Several studies have pointed to factors that make TYLCV and its whitefly vector well-suited to international travel. Firstly, whiteflies are relatively highly adept at surviving on plant materials during long-distance transport (Caciagli, 2007). At an upper limit of 30-40ºC adult whiteflies can only survive ~6 hours, but of course very few plant materials are transported under those conditions. At the lower limit, adult whiteflies have been shown to survive up to 4 days at 6ºC. Tomatoes, by comparison, are typically shipped between 10 and 20ºC (Suslow and Cantwell, 2009). Juvenile whiteflies can survive even longer at low temperatures – nymphs can survive at least 8 days at 4ºC, and eggs are still able to hatch after storage at 6ºC for 8 days. While most studies have not found TYLCV to be transovarially transmitted from parent whiteflies to progeny, at least one study has (Ghanim et al., 1998), suggesting the possibility that even if transport conditions are prohibitive for adult whiteflies, juveniles or eggs might be able to carry the disease during long-distance travel.

It has also been shown that whiteflies can acquire TYLCV inoculum directly from tomato fruit (Delatte et al., 2003). On Réunion Island, an overseas department of France approximately 800 km east of Madagascar, over 50% of imported tomato fruits coming primarily from Spain and Morocco were found to carry significant levels of virus inoculum. Furthermore, it was shown that whiteflies could acquire TYLCV by feeding on infected fruit, and could then transmit the virus to healthy plants. There are currently no international controls on the transport of tomato fruit from TYLCV-infected areas. As a result, it seems highly plausible that, even if the movement of planting materials is regulated, TYLCV can be transported internationally with produce imports.

Conclusions

The capacity of begomoviruses to acquire and recombine genetic diversity, and the polyphagous nature and high fecundity of the virus's whitefly vector, are both perfectly suited to take advantage of the homogenous agricultural environments that have become the norm across the globe in the last several decades. Furthermore, human beings have helped by transporting the virus and the vector from one location to another. The result has been the development of an unprecedented global pandemic in which the vast majority of tomato-producing regions of the world have faced decreasing yields and the need to apply protective measures to control the spread and the effects of TYCLV. The following section describes various control methods for TYLCV that have been developed since its emergence.

II. *Methods for the control of TYLCV*

Given the worldwide prominence of TYLCV, it is no surprise that significant efforts have been invested in its control. Earlier efforts focused primarily on physical and chemical barriers to prevent whiteflies from accessing plants, and more recently cultural practices for decreasing viral inoculum load in fields have shown serious success. The primary focus of TYLCV control efforts today is the breeding of tomato varieties with resistance to either the virus or the whitefly vector.

Physical and Optical Barriers

The most straightforward methods for controlling the spread of TYLCV involve those that place a physical barrier around growing tomato plants, thereby preventing whiteflies from accessing the plants (Polston and Lapidot, 2007). One common approach is to grow the tomatoes in net houses constructed of whitefly-proof 50-mesh screen material. These net houses provide extremely effective whitefly control, and can limit whitefly populations to just 1% of those observed in greenhouses lacking whitefly-proof screens (Berlinger and Lebiush-Mordechi, 1995). Unfortunately these screens are not sufficient to prevent TYLCV infection on their own as small numbers of whiteflies can gain access to the net houses through gaps in the screening material around entrances and by clinging to personnel. However, in conjunction with infrequent pesticide applications TYLCV can be completely controlled in these structures. The downsides of using such fine-mesh net houses are that they are expensive and can create problems of shading, overheating, and poor ventilation. In mid-summer these conditions can lead to heat-stress on tomatoes, affecting fruit set and quality. In addition poor ventilation can lead to increased humidity and enhance the spread of foliar diseases. Positive pressure ventilation systems have been used to overcome the overheating and ventilation problems, and in addition increase the

whitefly-exclusion properties of the net houses (Weintraub and Berlinger, 2004), but add even more to production costs.

An alternative whitefly exclusion method uses ultraviolet-absorbing plastic films for the construction of greenhouses or hoop-houses (tunnels) (Antignus et al., 1996). These films, which consist of a regular greenhouse polyethylene film impregnated with a UV-blocking material, allow 80% transmission of light in the visible range (380 – 700 nm) but only 5% transmission in the UV range (280 – 380 nm). In contrast, regular polyethylene films allow 13-20% UV transmission. This decrease in UV light transmission can play a significant role in preventing whitefly infestations. Unlike humans, insects can perceive ultraviolet (UV) light, and it plays an important role in various aspects of their behavior, including orientation, navigation, feeding, and mating. Feeding behavior in whiteflies involves a chain of events that begins with the whitefly orienting towards the plant from a distance (Byrne and Bellows, 1991). The exclusion of UV light from the growing area essentially blinds whiteflies, inhibiting their ability to find host plants upon which to feed. Greenhouses with UV-absorbing plastic films have been observed to have as much as an 80-fold reduction in TYLCD incidence (Antignus, 2007). However, like net-houses, UV-blocking greenhouses have problems of overheating and poor ventilation. Positive-pressure ventilation systems can improve performance, but again at an increased financial expense that is not always feasible.

Optical barriers to whitefly access also exist for field-grown tomatoes, in the form of reflective mulches. Yellow plastic mulches have been in use since the 1960s (Nitzany et al., 1964), when it was discovered that the color yellow may be a component of the whitefly's host-selection mechanism (Mound, 1962). (This correlates well with the

tendency for begomoviruses to induce yellowing symptoms.) Yellow mulches appear to disorient whiteflies by inhibiting their ability to accurately discern host plant leaves against the yellow background. As a result, a significant portion of whiteflies are attracted to the mulch rather than the plant – in the hot, arid environments common to many tomato-growing regions the whiteflies then typically die of overheating and desiccation within one hour (Cohen, 1982). The effectiveness of yellow mulches is inversely correlated with the density of the plant foliage – as plants mature and their foliage covers a significant portion of the mulch, the amount of reflected yellow light is diminished until it becomes ineffective. However, since the impact of TYLCD is greatest when plants are infected early in development, the 20 – 30 days of protection offered by yellow plastic mulches has a serious impact on the losses from the disease. In one study, at 38 days after transplanting only 10% of the tomato plants protected by a yellow mulch showed TYLCD symptoms, as compared with 100% of the control plants (Cohen and Melamed-Madjar, 1978).

Interestingly, yellow mulches have limited effectiveness in humid climates, where whiteflies are not quickly dehydrated by the high heat of the mulch (Csizinszky et al., 1999). In such cases, highly reflective aluminized mulches have been found to be significantly more effective. While the mechanism of the protection offered by aluminized mulches in humid climates is not fully understood, it is believed that the high reflectivity of both visible and UV light has a more significant disorienting effect on whiteflies, and thus is more effective at preventing their landing on plant leaves once they survive their initial landing on the mulch. Both yellow and aluminized mulches offer an inexpensive and effective method of whitefly control in developed countries, but lack of access in the developing world often make them prohibitively expensive.

Chemical Control of Whiteflies

While physical and optical barriers provide significant protection from whiteflies, they are often used in conjunction with chemical methods of whitefly control for more complete reduction of disease incidence. A number of chemical classes have been used to reduce whitefly populations including chlorinated hydrocarbons, organophosphates, neonicotinoids, pyridine-azomethines, and pyrethroids (Polston and Lapidot, 2007). Over time, repetitive and frequent use of these pesticides has led to resistant whitefly variants in many locations (Palumbo et al., 2001), in addition to resistant secondary pests such as leafminers (Polston and Lapidot, 2007). In the decade since their introduction, neonicotinoid insecticides such as thiamethoxam, imidacloprid, and dinotefuron have become particularly popular due to their narrow targeting of sucking insects such as whiteflies, aphids and leafhoppers and their consequently mild effect on beneficial insects that act to control pest insect populations in the field (Ahmed et al., 2001; Mason et al., 2000). These insecticides, which have a low toxicity to mammals, are taken up by plant roots and can be found systemically throughout plants, typically limiting their impact to those insects that ingest plant tissues or sap (Jeschke and Nauen, 2008). However, their extensive use has led to increasing incidence of neonicotinoid resistance in whiteflies in several places around the world (Cahill et al., 1996; Elbert and Nauen, 2000). Today, the exclusive use of insecticides and other chemical insect controls such as insect growth regulators, oils, and soaps is considered insufficient for the control of TYLCD, as whiteflies are sufficiently fecund, mobile, and resistant to chemicals to serve as an effective begomovirus vector even when pesticides are applied three times per week (Antignus, 2007). However, in conjunction with other protective methods chemicals

do provide a further layer of protection to prevent whiteflies from spreading TYLCV from one plant to the next.

Cultural Practices

While physical and chemical approaches can offer significant reductions in whitefly populations and therefore significant reductions in the spread of TYLCV, there are several cultural practices that have been shown to be effective in reducing the incidence of the virus without requiring any specialized inputs. One such approach is the use of bait crops, in which preferred hosts are offered to attract whiteflies away from tomatoes, and another is the use of a host-free period to clear viral inoculum from the area prior to the planting of a host crop.

Bait Cropping

Bait cropping is a means of slowing the accumulation of viral inoculum in the regional host and vector populations by offering a crop for whitefly feeding that is an attractive host for the insect but is a non-host for the virus (Antignus, 2007). Cucurbit species such as squash and cucumber have been effectively used to reduce infection rates of TYLCV in tomato. It has been shown that while whiteflies show no particular preference for landing on a tomato leaf or a cucurbit leaf, once they do land they are more likely to remain on the cucurbit leaf to feed (Cohen et al., 1988). A study in 1982 in which rows of tomato were planted alternately with rows of cucumber that had been planted 30 days earlier showed that the development of TYLCV symptoms on the tomatoes was delayed by almost two months compared with a control plot (Al-musa, 1982). Unfortunately, intercropping is a labor-intensive process that does not integrate well with today's mechanized approaches to large-scale agriculture. However, a more recent study from 2004 showed that tomatoes in a plot neighboring a plot of squash

had fewer instances of TYLCV infection than those in a plot neighboring other tomato plots (Schuster, 2004). Thus, while bait cropping might not be effective as an exclusive method of TYLCV control, it does help to reduce disease pressure, particularly in smaller-scale situations.

Host Free Period

The strength of the viral disease pressure on field-grown host plants depends not only on the population of the insect vector, but also on the quantity of viral inoculum accumulated in the local host and vector populations. Any means of reducing the viral load in the system, whether or not it also reduces vector populations, should be effective at reducing the disease pressure incident on the local cultivated host population. The host-free period is a community-based approach to reducing both local vector populations and overall viral load by removal of all host plants for a set period of time during an agreed-upon off-season. While not particularly common due to its requirement for communal organization and participation, the use of a host-free period has successfully reduced the significance of TYLCV in Cyprus (Ioannou, 1987), Israel (Ucko et al., 1998), and the Dominican Republic (Salati et al., 2002).

The basic logic of the host-free period starts with the understanding that begomoviruses are not passed transovarially from parent whiteflies to their progeny, and that the whitefly adult lifespan is only approximately one month long (Byrne and Bellows, 1991). It stands to reason that if a community of growers can prevent all whiteflies from taking up new viral inoculum for one month then all viruliferous whiteflies will die and all viral inoculum will be flushed from the system. During the host-free period, all farmers in a region agree to abstain from growing any hosts of the begomovirus in question. During that time period, viruliferous whiteflies may find

hosts to feed upon, but those hosts will not be susceptible to the virus and therefore will not allow the virus to replicate and create a new pool of inoculum. Over the course of several weeks, all whiteflies carrying the virus will die, and thus by the end of the host free period the whitefly population will be completely free of viral inoculum. In the weeks following the host-free period, when virus-susceptible seedlings are transplanted to the field, the virus pressure will be low enough to allow the seedlings to mature to a stage where they can better withstand a TYLCV infection without losing their entire yield. Of course, unless the region practicing the host-free period is exceedingly isolated, TYLCV will eventually be re-introduced from a neighboring region, but the reduced pool of viral inoculum at the start of the season, when the host plants are the most susceptible, can have a serious impact on yield.

There are several factors that affect the success of a host-free period. Firstly, 100% cooperation is important. A single farmer who chooses to break the rules can cultivate a tremendous reservoir of viral inoculum in his field, negating the efforts invested by others. Most communities that adopt a host-free period impose harsh penalties to prevent violations – in the Dominican Republic, for instance, violators of a legally-enforced host-free period in June, July and August are subject to having all of the plants in their fields immediately destroyed upon discovery. Secondly, it is very important for a community to understand the local ecology and epidemiology of the virus, which requires knowing which cultivated and wild species can serve as a host for the virus. This includes species that act as symptomless hosts, which allow significant accumulation of viral inoculum without showing any perceptible outward symptoms (Salati et al., 2002). With a quickly-evolving virus it is important to continuously monitor the viral host range in order to adapt to any changes that arise. For example, farmers in southern Spain have traditionally only grown tomatoes during

the warmer summer months, and have therefore had a natural host-free period during the winter months. When TYLCSV was first introduced to the region in the early 1990s, this fact helped to control the spread of the virus. However, when TYLCV-IL was introduced in the late 1990s, it came with a slightly different host range that worked to its significant advantage – it could replicate in common bean (*Phaseolus vulgaris*), which is regularly grown through the cooler months in southern Spain (Sanchez-Campos et al., 1999). As a result TYLCV quickly increased in prevalence and surpassed TYLCSV as the dominant tomato-infecting begomovirus in the region (Moriones and Navas-Castillo, 2008). Finally, it is important for those engaging in a host-free period to practice very thorough sanitation methods in their fields. At the end of the growing season, all tomato plant residues must be destroyed, preferably by some form of cultivation such as deep plowing or disking, to prevent any leftover materials from remaining as viable hosts for the virus during the host-free period. Old tomato plants left to languish in the field allow viral replication, but are undesirable whitefly hosts which encourage the dispersal of viruliferous whiteflies to neighboring fields, thus encouraging the spread of the disease (Gilbertson et al., 2007). In addition to old plants, volunteer host plants must be removed during the host-free period to prevent the spread of viral inoculum.

The most successful account of a host-free period for the control of TYLCV began in the Dominican Republic in 1995. In the late 1980s the Dominican Republic had a flourishing tomato processing industry with ~8000 hectares under cultivation and producing enough tomato paste to satisfy the needs of the ~7-8 million inhabitants of the country (Gilbertson et al., 2007). However, in 1992 tomato transplants were imported from foreign sources after local seedling nurseries failed to produce viable transplants due to heavy rain. Some of the foreign tomato transplants were likely

infected with TYLCV, as the disease quickly established itself on the island during the 1992-1993 growing season (Nakhla et al., 1994). By the following year the disease had become so pervasive and damaging that tomato yields were estimated to have decreased by 75%, leading to losses estimated at over US$10 million as well as the need to import over 16,000 tons of tomato paste. A host-free period was established during June, July and August of 1995 for a variety of local vegetable crops including not only tomato, bean and pepper, which can all function as hosts for at least some TYLCV strains, but also cucurbits, eggplant, and okra, which are TYLCV non-hosts but are favored hosts for whiteflies (Salati et al., 2002). The host-free period was remarkably successful in reducing the TYLCV disease pressure during the 1995-1996 growing season, likely due at least in part to government enforcement of the host-free period. As a result, farmers in the Dominican Republic began to accept the host free period as part of their yearly routines, and voluntary compliance greatly increased in subsequent years. The host-free period is still in use today, and monthly whitefly monitoring since 1997 has shown a consistent pattern in which virus levels in the local whitefly population drop dramatically going into the host-free period, and stay low through September, October, and often the first half of November as well. Though levels then typically increase dramatically, often leading to 100% infection rates in January and February, the continuity of the decrease in viral inoculum levels into the beginning of the growing season provides farmers with a significant head start against the disease, and has allowed the tomato industry of the Dominican Republic to again become self sufficient (Gilbertson et al., 2007).

Breeding for Resistance to TYLCV

Given the limited success and/or high costs (in both material and time) of the various cultural, chemical, and physical approaches to the control of TYLCV, it has been clear

from early on in the disease epidemic that the best hope for successful control of the virus lies in the development of tomato cultivars that are resistant to the disease. Resistance entails a range of possible reactions, from immunity, in which a plant functions as a non-host, allowing no viral replication, to tolerance, in which the plant allows viral replication and shows some level of disease symptoms but nonetheless has high fruit set and quality. Efforts to identify TYLCV-resistant tomato varieties began in the 1960s with several screens of popular cultivars, but the results were uniformly negative (Pilowsky and Cohen, 1974). It has since been determined that all known tomato cultivars (species *Solanum lycopersicum*) are susceptible to TYLCV (Ji et al., 2007b).

In the 1970s plant breeders began turning to wild species closely related to tomato in an effort to identify begomovirus resistance genes. Wild *Solanum lycopersicum*, the ancestor of cultivated tomato, is just one of ten recognized species that make up *Solanum* L. section *Lycopersicon*, the wild tomatoes (Spooner et al., 2005). All wild tomato species are endemic to western South America, with a distribution stretching from Ecuador to northern Chile. Eight of the species can be found along the coast and in the Andes highlands, while two more are native to the Galápagos Islands. The wild tomato species have a variety of mating systems ranging from autogamous self-compatible to facultative allogamous self-compatible to allogamous self-incompatible. All can be crossed to cultivated tomato, though often with difficulty. Given the low level of genetic diversity available within *S. lycopersicum*, though, wild tomato species have become an important source of genetic material for plant breeders and have been used extensively as sources of disease resistance and agronomic traits (Bai and Lindhout, 2007).

To date TYLCV resistance has been identified in *Solanum cheesmaniae*, *Solanum chilense*, *Solanum habrochaites*, *Solanum peruvianum*, and *Solanum pimpinellifolium*. In each case resistant accessions were identified in screens in which many (sometimes thousands) of accessions were evaluated. While protocols differ from one study to the next, these resistance screens often involve the controlled inoculation of tomato plants by incubation in a greenhouse or insect-proof net house containing viruliferous whiteflies (Lapidot, 2007). The whiteflies are maintained in a separate net house on a preferred host, such as cotton (*Gossypium hirsutum* L.), and the viral culture is maintained in yet another net house on a susceptible host such as cultivated tomato or *Datura stramonium*. Whiteflies are raised on the preferred host, incubated on the susceptible host for a set period of time for the acquisition of the virus, and then transferred to the enclosure containing the test plants for inoculation. Typically several plants of each accession are tested, since accessions represent collections from a single location but cannot be guaranteed to be genetically homogenous, and in allogamous species such as *S. peruvianum* and *S. chilense* can be almost guaranteed to be heterogeneous. In some screens, especially earlier ones, plants were field grown and inoculation was by local populations of viruliferous whiteflies.

The identification of accessions of a wild tomato species resistant to TYLCV is often followed by an attempt to introgress the resistance trait into a cultivated tomato variety, a challenging endeavor due to various barriers inhibiting the success of an interspecific cross (Pico et al., 2002) and the existence of wild, undesirable traits that can be carried along with the disease resistance trait by linkage drag (Gur and Zamir, 2004). While wild and cultivated tomato species can technically be crossed, oftentimes special techniques are required to promote seed set. In almost all cases, the wild species must be used as the pollen donor, as cultivated pollen will rarely lead to seed

set in a wild individual (Hogenboom, 1972). In cases with only mild crossability barriers, mixtures of wild and cultivated pollen can be used to ensure fruit set following pollination (Philouze, 1967). In more severe situations, however, embryo abortion occurs, leading fruit to set but with no viable seeds (Rick and Butler, 1956). To overcome this barrier embryo rescue techniques are used, in which the nascent embryo is removed from a seed and grown in culture (Stewart, 1981). Even in cases that result in successful hybrids, undesirable wild traits that are difficult to remove are often found in the progeny. Most wild tomato species lack some of the basic traits of the tomato domestication syndrome, including both consumer-oriented traits such as large, tasty, red fruit, and grower-oriented traits such as compact growth habit and earliness. In interspecific hybrids between wild and cultivated tomatoes, inhibition of recombination due to differences in genomic sequence often leads to the existence of large linkage blocks that might contain both the trait of interest (e.g. TYLCV resistance) and undesirable traits (Bai and Lindhout, 2007). Breaking those linkage blocks is a significant challenge that plant breeders address when introgressing desirable traits from a wild species of tomato.

In the last three and a half decades extensive efforts have been invested in the development of TYLCV-resistant tomato cultivars through the identification of novel resistance sources, the generation of resistant breeding lines, and the identification of molecular markers linked with resistance genes. Below is a summary of all of those efforts organized by resistance source.[1]

[1] Until a recent reevaluation of the phylogeny of genus *Solanum*, all tomato species were considered to be members of the genus *Lycopersicon*, and thus are often referenced in the literature by their former names (Spooner, Peralta et al. 2005). For that reason, synonyms of each species name are provided in the heading of each subsection below. The synonym for *Solanum lycopersicum* is *Lycopersicon esculentum*.

Solanum pimpinellifolium (syn. *Lycopersicon pimpinellifolium*)

The first major effort to identify accessions of wild tomato with TYLCV resistance was conducted by Pilowsky and Cohen of the Israeli ARO in 1974. They focused their efforts on *S. pimipnellifolium* (common name: currant tomato) since of all the wild tomatoes it is the most compatible for crossing with cultivated tomato. Through their screens they identified several resistant accessions showing only moderate viral accumulation and strongly attenuated symptoms. This included accession LA 121, which they crossed with *S. lycopersicum* to study its inheritance. Genetic analysis of $F_1 – F_3$ and backcross generations indicated the incomplete dominance of resistance over susceptibility, which they suggested implied monogenic control of resistance (Pilowsky and Cohen, 1974). Despite the strong TYLCV resistance shown by LA 121, tomato breeding lines descended from it had moderate disease symptoms but significantly decreased vigor and yield, resulting in the termination of this effort.

Further accessions of *S. pimpinellifolium* were screened by Geneif in Sudan in 1977-1982, along with accessions of *S. peruvianum*, *S. habrochaites* and *S. lycopersicum* (Geneif, 1984). *S. pimipnellifolium* accession LA 1478 was identified as a good source of TYLCV resistance and a strong performer in the hot and dry environment of Sudan, and was therefore crossed with locally popular commercial cultivars including Money Maker and Early Pack to generate TYCLV resistant cultivars. Inheritance studies showed a clear 3:1 ratio of resistant to susceptible phenotypes in the F_2 generation, and backcross progenies segregated in a 1:1 ratio, implying that resistance was controlled by a single dominant gene.

In 1988 and 1989 Kasrawi et al. of the University of Jordan evaluated numerous accessions of several different tomato species, including both LA 1474 and the

accession hirsute INRA of *S. pimpinellifolium*. Both were shown to have strong TYLCV resistance (Kasrawi et al., 1988) which was conditioned by a single dominant gene, designated *Tylc* (Kasrawi, 1989).

In 1997 a research study was conducted to identify the locus responsible for TYLCV resistance in the hirsute INRA (Chagué et al., 1997). A TYLCV-resistant tomato breeding line containing introgressions from hirsute INRA was crossed with a susceptible breeding line followed by several rounds of selfing to generate F_4 lines. Thirty individuals from each line were evaluated for TYLCV resistance by agroinoculation, and the five most resistant and six most susceptible lines were used for Bulk Segregant Analsysis. Four random amplified polymorphic DNA (RAPD) markers, all mapping to an introgression of 17.3 cM on chromosome 6, were reported to be linked to the resistance trait.

Solanum peruvianum (syn. *Lycopersicon peruvianum*)

One of the first major resistance screens to look at accessions of *S. peruvianum* as well as *S. habrochaites, S. cheesmaniae* and a large selection of commercial cultivars was conducted by a team at the University of Cairo in Egypt starting in 1980 (Hassan et al., 1982). Greenhouse inoculated plants were scored on a symptom severity scale of 0 to 4. While none of the commercial cultivars showed any virus resistance, all accessions of *S. peruvianum* showed almost no symptom development. Of the *L. peruvianum* accessions identified in this screen, the most important to emerge was CMV sel INRA, which was crossed with the locally popular cultivar Mortelglan using the mixed pollen technique (Hassan et al., 1984). 3 F_2 plants were found to be free of virus symptoms and were propagated with selection to the F_4 generation, at which point all plants were found to be resistant to TYLCV. F_5 seeds were collected and sent

to the French National Institute for Agricultural Research (L'Institut National de la Recherche Agronomique, INRA) where a gene pyramiding breeding project was conducted (described in detail below).

The first commercially available TYLCV-resistant line, TY-20, was released in Israel in 1988. Designated a tolerant variety, TY-20 derived its resistance from the *S. peruvianum* accession PI 126935. When infected with TYLCV, young TY-20 plants did show mild interveinal chlorosis, and older plants did display minor leaf cupping, but plants were nonetheless able to give a decent yield (Pilowsky and Cohen, 1990). Evaluation of the genetics of the TYLCV tolerance derived from PI 126935 indicated that the trait was controlled by five recessive genes. Virus transmission tests additionally showed that infected TY-20 plants were as effective at transmitting the virus as popular susceptible cultivars (Pilowsky and Cohen, 1990), though later tests showed that the virus titer in TY-20 was only ~50% of that in various susceptible cultivars (Rom et al., 1993). Given TY-20's complicated genetics and its poor ability to slow the spread of TYLCV, researchers quickly sought better resistance sources.

Continued screening efforts at the ARO in Israel identified several accessions of *S. peruvianum* with stronger levels of resistance: PI 126926, PI 126930, PI 390681, and LA 441. These four accessions were each crossed with susceptible cultivated tomato line 1630 using the mixed pollen approach, and after many rounds of backcrosses, sib crosses, and phenotypic evaluation, a single highly resistant individual showing a symptomless reaction to TYLCV was selected and its bulked offspring were designated line TY172 (Friedmann et al., 1998). When compared with plants of susceptible and tolerant cultivars in controlled inoculations, TY172 had only very minor yield losses under disease pressure, and showed significantly reduced

accumulation of viral transcript (Lapidot et al., 1997). When TY172 was crossed with a susceptible individual, the resultant F_2 population had a mix of plants showing mild symptoms in response to TYLCV in addition to individuals with symptomless responses. The ratio of symptomless to mildly-symptomatic plants was measured as approximately 7:64, implying that TYLCV resistance in TY172 is controlled by at least three genes (Lapidot et al., 2000).

Solanum chilense (syn. *Lycopersicon chilense*)

A team at the Hebrew University in Jerusalem conducted of a field-based screen of 23 tomato accessions representing several different wild tomato species in the Jordan River Valley in 1988. One accession of *S. chilense*, LA 1969, stood out as an extremely promising source of resistance, showing no TYLCD symptoms and almost no viral accumulation (measured by squash blot hybridization) as long as 84 days after planting, in a region that routinely sees 100% infection rates (Zakay et al., 1991). Similar results were subsequently obtained following inoculation with viruliferous whiteflies in a greenhouse. An evaluation of LA 1969 in Florida in 1990 showed it to be resistant to the locally emerging tomato begomovirus, likely *Tomato mottle virus*, ToMoV (Scott and Schuster, 1991), and a similar study in Taiwan in 1994 additionally showed the accession to be resistant to *Taiwan tomato yellow leaf curl virus*, TTYLCV (Chiang et al., 1994).

With the strength of the begomovirus resistance in LA 1969 firmly established, a breeding program was initiated at Hebrew University to introgress the trait into cultivated tomato. In 1988 Zamir et al. crossed LA 1969 with tomato cultivar M82-1-8 to obtain a population of interspecific hybrids (Zamir et al., 1994). Due to the strong crossability barriers between *S. lycopersicum* and *S. chilense* only one single

interspecific hybrid was produced from 300 pollinated flowers, and when that one plant was crossed as a male parent back to *S. lycopersicum*, only five fertile BC_1 plants were obtained from the 100 fruits set. However, two of those five plants were found to be TYLCV-resistant, and they were selfed to generate BC_1S_1 populations for a typical backcross breeding program. Populations were phenotyped in three different locations in Israel and/or in controlled inoculation setups in greenhouses, and the most resistant individuals were selected for further rounds of backcrossing and selfing. What differentiates this breeding program from previous efforts to breed TYLCV-resistant tomato cultivars is that restriction fragment length polymorphism (RFLP) molecular markers were used to correlate chromosomal segments with TYLCV resistance, allowing the later stages of the breeding program to be accelerated as genotyping could help narrow the pool of individuals needing phenoytping. Resistance genes were roughly mapped in the BC_2S_1 population to chromosomes 6, 3, and 7, and finely mapped in the BC_3S_1 population. The TYLCV-resistance trait was found to be associated with a partially-dominant gene located on chromosome 6, which was assigned the name *Ty-1*, and two modifier genes on chromosomes 3 and 7. Since the breeding program yielded not only breeding lines nearly isogenic to *S. lycopersicum* with introgressions for TYLCV resistance, but also molecular markers associated with the introgressed TYLCV-resistance genes, *Ty-1* has become an extremely popular source of TYLCV resistance and has been incorporated into many tomato breeding programs in the last decade.

Concurrently with the breeding efforts at Hebrew University, a breeding program working with *S. chilense* was initiated in Florida in 1990 with the specific goal of developing tomato cultivars with resistance to the bipartite begomovirus ToMoV (Scott et al., 1995). A screen of 23 *S. chilense* accessions showed all to have

significant ToMoV resistance, so the 12 with the fewest confounding features were selected for the breeding program. (For example, smaller-leaved plants were excluded due to the possible confounding effect of less surface area for whitefly feeding.) Crosses with a cultivated tomato breeding line yielded 597 fruit but just 15 F_1s, ten from true seed and five from embryo rescue. Subsequent efforts were made to maintain the genetic diversity in the breeding pool through early generations by extensively using embryo rescue to prevent the exclusion of any genetic combinations with superior disease resistance but poor germination ability. The initial backcross generation contained only 43 BC_1 plants, but following embryo rescue that number increased to 555. Subsequent rounds of selection, backcrossing and selfing narrowed the pool to descendents of just 6.3% of the original 555 BC_1 plants. Homozygosity for ToMoV resistance began to be observed by the $F_1BC_1S_4$ or $F_1BC_2S_3$ generation, at which point 37,000 plants had been screened for virus resistance. The final selection included 12 lines representing five resistance sources: LA 1932, LA 1938, LA 1961, LA 1968, and LA 2779. These 12 lines were sent to the Dominican Republic for TYLCV resistance trials, where they were shown to carry resistance not only to ToMoV but also very strongly to TYLCV-IL[DO], with 9 of the 12 varieties performing significantly better than TY-20.

Since the introduction of these begomovirus resistant breeding lines, those derived from LA 1932 and LA 2779 have shown to provide the strongest resistance and have been commonly used in breeding programs throughout the world. Several efforts have been made to map the resistance genes in these lines with successively finer resolution, providing breeders with molecular markers to accelerate their breeding pipelines. Inheritance studies and QTL mapping using RAPD markers indicated that three loci on chromosome 6 are responsible for resistance to both ToMoV and

TYLCV (Agrama and Scott, 2006; Griffiths and Scott, 2001). More recently, a study using sequence-characterized amplified region (SCAR) markers further refined the location of the major gene controlling TYLCV resistance and designated it *Ty-3* (Ji et al., 2007a). *Ty-3* has been found to be responsible for approximately 65% of the TYLCV resistance in lines derived from LA 1932 and LA 2779. Since it is not possible to concurrently score for resistance to TYLCV and ToMoV in the same plant, the mapping of resistance to the two begomoviruses could not be performed in the same mapping population, but the major factor for ToMoV resistance did map to the same location, implying that *Ty-3* may be responsible for both resistances.

Though they are on the same chromosome arm, it has been determined that *Ty-1* and *Ty-3* are unlikely to be allelic for a variety of reasons (Ji et al., 2007a). Firstly, while the introgression on the long arm of chromosome 6 from *S. chilense* in lines derived from LA 2779 does overlap with the region containing *Ty-1*, the introgression in lines from LA 1932 does not. Similarly, the introgression in many *Ty-1* lines derived from LA 1969 does not overlap with the *Ty-3* locus. In fact, the map locations for *Ty-1* and *Ty-3* are approximately 15 cM apart. Additionally, *Ty-1* does not confer resistance to ToMoV, while *Ty-3* does. Finally, *Ty-1* has been shown to be almost completely dominant, while *Ty-3* confers equal contributions to TYLCV resistance from dominance and additive effects. Given these facts, it seems likely that *Ty-1* and *Ty-3* are two separate genes, which raises the possibility that they could be combined for even greater resistance. Unfortunately the two genes are linked in *trans*, and since genomic sequence divergence tends to inhibit recombination, it will take more time and effort for breeders to break those linkages. The availability of tightly linked molecular markers will help, however, by allowing breeders to screen plants at an

early stage, saving both time and space and allowing breeders to generate many more plants with potential recombination events.

Solanum habrochaites (syn. *Lycopersicon hirsutum*)

In addition to *S. peruvianum*, several accessions of *S. habrochaites* were also evaluated at the University of Cairo in 1980 for TYLCV resistance (Hassan et al., 1982). Of the seven resistant accessions identified, LA 386 proved to be the most highly resistant. Meanwhile, a screening program in Cyprus in 1985 also reported several resistant accessions of *S. habrochaites*, including LA 1777 (Ioannou, 1985). The resistance of these two accessions was confirmed several times in the intervening years (Hassan et al., 1984; Zakay et al., 1991), but it was not until 1998 that a breeding program attempted to use them as a resistance source. In that year a breeding team at Hebrew University crossed the two accessions, yielding highly resistant F_1 hybrids. These F_1 hybrids were then crossed twice with *S. lycopersicum*, with embryo rescue being necessary following the first cross to generate viable progeny. Rounds of phenotypic evaluation, involving whitefly-mediated inoculation in greenhouses, were alternated with rounds of selfing until fixed TYLCV resistant lines were obtained in the BC_1F_4 generation (Vidavsky and Czosnek, 1998). In these lines, inheritance studies implied a single dominant factor associated with tolerance (i.e. ability to perform well despite infection by the virus) and two or three recessive genes associated with immunity (i.e. prevention of the accumulation of viral transcript). One particular breeding line from this program, Ih902, has become a popular source of TYLCV resistance.

A separate line of work with *S. habrochaites* was initiated concurrently with Hassan's screens in 1982 at Haryana Agricultural University in India. A series of screens there

identified a number of *S. habrochaites* accessions resistant to *Tomato leaf curl virus* (ToLCV), a tomato-infecting begomovirus endemic to India. One accession in particular, *S. habrochaites* f. *glabratum* B6013, exhibited very promising resistance, showing no symptoms at all during the screen (Banerjee and Kalloo, 1987a). Inheritance studies showed that the resistance trait of B6013 is controlled by two epistatic genes (Banerjee and Kalloo, 1987b). Accession B6013 was crossed with several locally popular tomato cultivars in 1982, and F_1s were phenotypically evaluated for ToLCV resistance by controlled inoculation with viruliferous whiteflies in a greenhouse. Resistant individuals were backcrossed to their cultivated parents, and the procedure was repeated until the BC_6 generation, at which point lines were observed to be fixed for ToLCV resistance (Kalloo and Banerjee, 1990).

While the breeding lines from Haryana Agricultural University were subsequently found to carry resistance to only some tomato-infecting begomoviruses, they were very effective throughout much of Asia including south India, Taiwan, Japan, and north Vietnam, and were therefore were used extensively by the Asian Vegetable Research and Development Center (AVRDC, now known as the World Vegetable Center) in its breeding program in Taiwan. (Line H24 in particular was a very popular source of resistance.) Researchers at AVRDC mapped the resistance trait using RFLP markers in a collection of F_3 families from a cross between H24 and a susceptible tomato cultivar, and found it to be associated with a single introgression of 14.6 cM on the short arm of chromosome 11 (Hanson et al., 2000). Formally named *Ty-2* (Hanson et al., 2006), the gene has been further mapped to a 10 cM region surrounding a single PCR-based molecular marker (Ji et al., 2007b).

Pyramiding resistance genes

At this point in time, TYLCV resistance genes have been identified in *S. pimpinellifolium, S. peruvianum, S. chilense,* and *S. habrochaites,* and each source has yielded breeding lines that offer strong protection against TYLCV in the field. While it has been argued persuasively that *Ty-1* and *Ty-3* are not at the same locus, it remains to be seen whether any of the other resistance genes identified in different species actually represent the same locus. In that vein, several recent observations are enlightening. An analysis of TY197, a line deriving from the work with *S. peruvianum* at the ARO in Israel (Lapidot et al., 1997), has shown that the line does not carry introgressions from *S. peruvianum* corresponding to the *Ty-1*, *Ty-2*, or *Ty-3* loci (Ji et al., 2007b). One of the four markers mapped to the introgression responsible for TYLCV-resistance in a *S. piminellifolium* hirsute INRA-derived line (Chagué et al., 1997) has more recently been found to map very closely to *Ty-3*, near 25 cM on chromosome 6 (Ji et al., 2007b). It is therefore possible that these genes are allelic. Finally, cultivars in Guatemala deriving their resistance from the Israeli *S. habrochaites*-derived breeding line Ih902 (Vidavsky and Czosnek, 1998) have been shown to have many identical sequences to *S. chilense* LA 2779-derived varieties in the 13 cM to 32 cM range of chromosome 6, which contains *Ty-3* (Ji et al., 2007b), implying the possibility that *Ty-3* is also responsible for some *S. habrochaites*-derived resistance.

As the collection of successful TYLCV-resistant breeding lines grows, attention in the breeding community is beginning to shift towards the pyramiding of multiple TYLCV resistance genes in a single cultivar. The earliest work in this vein was conducted by an international team of researchers let by H. Laterrot of INRA in Avignon, France in the mid-1990s (Laterrot, 1995). Various breeding populations generated from

interspecific crosses between a resistant accession of a wild species and a susceptible cultivated line were sent to collaborators in locations with strong TYLCV pressure including Cyprus, Egypt, Israel, Jordan, Lebanon, Mali, Senegal, Sudan, and Turkey. Populations were screened in each location, and half the seeds from the selected individuals were sent back to INRA, while the other half were used as the resistance sources in local breeding programs. (Hence the work of Hassan et al. with *S. peruvianum* in 1984.) Partners were also free to share their breeding lines with INRA. Each year, selections and breeding lines representing two different resistance sources were combined to generate a single population with multiple resistance genes. Thus, the Chépertylc 92 population was bred in 1992 from selections from two populations, one with *S. cheesmaniae* LA 1401 as a resistance source, and the other using *S. peruvianum* CMV selection INRA. Similarly, Pimpertylc 93 was derived from populations based on *L. pimpinellifolium* Hirsute INRA and *L. peruvianum* CMV selection INRA. It is not clear if these materials were successfully bred into commercial cultivars, but at the very least they provided a very solid theoretical foundation for further work in pyramiding resistance genes.

More recent work in pyramiding resistance genes has been conducted by Vidavsky et al. (2008), who have begun making diallele crosses between one susceptible breeding line and six resistant lines representing *S. pimpinellifolium* (line PIMHIR, derived from accession Hirsute INRA, Laterrot, 1992), *S. peruvianum* (lines TY172, Friedmann et al., 1998; and TY197, Lapidot et al., 1997), *S. chilense* (lines Fla-595-2, Griffiths and Scott, 2001; and TY-52, Zamir et al., 1994) and *S. habrochaites* (line Ih902, Vidavsky and Czosnek, 1998). Progeny of these crosses have shown that hybrids between a resistant breeding line and a susceptible breeding line are always more TYLCV resistant than the susceptible parent, and when two resistant breeding

lines are crossed the resultant hybrid is typically at least as resistant as the more resistant parent. Interestingly, the most highly resistant hybrid resulted from the cross between *S. habrochaites* Ih902 and *S. peruvianum* TY172, the former of which is thought to possibly contain *Ty-3* as well as several recessive factors conditioning immunity, and the latter of which has been characterized as having at least three genes responsible for TYLCV resistance (as described above).

Cultivars combining several different sources of TYLCV resistance promise a range of advantages over those derived from single sources. Cultivars derived from single sources of resistance have at times been found to offer resistance to only some of the many tomato-infecting begomoviruses responsible for TYLCD, and their resistance therefore breaks down in locations with different complements of viral strains (Hanson et al., 2000). By pyramiding resistance genes, a breeder can increase the odds that the same cultivar will offer a decent level of virus resistance in multiple locations throughout the world. Multiple resistance genes also often offer a stronger level of resistance than single genes, as they have the potential to interfere with multiple steps of the begomovirus infection cycle. Resistance to a wider range of viruses and stronger suppression of a range of viral capacities can work together to strongly decrease the evolution of the virus: as fewer viral strains infect the same plant, there are fewer opportunities for recombination events to yield new strains, and with a diminished capacity to undergo rounds of replication, the virus also has fewer opportunities to experience mutations. As a result, pyramiding resistance genes can strongly decrease the odds of a virus evolving to overcome the resistance trait, rendering cultivars combining multiple resistance sources very valuable resources in the fight against TYLCV.

Breeding for resistance to Bemisia whiteflies

While the majority of the breeding work aimed at decreasing the impact of TYLCV has focused on breeding for resistance to the virus itself, there has also been some work focused on breeding for resistance to the whitefly vector. Resistance to whiteflies would be equivalent to broad-spectrum resistance to all begomoviruses, and would thus be a very valuable trait in cultivated tomato.

Solanum pennellii has high densities of type IV glandular trichomes on all green above-ground tissues of the plant, and 90% of the exudates from these trichomes have been shown to be acylsugars. Acylsugars have a deterrence effect on a wide range of pests, including *Bemisia tabaci*. The deterrence effect manifests as a strong delay in feeding, with insects waiting longer before probing a leaf surface, and probing fewer times thereafter, than on leaves without strong concentrations of acylsugars. In addition, high acylsugar concentration strongly reduces oviposition by whiteflies. These deterrence effects can have a serious impact on the spread of whitefly-vectored viruses, as decreased probing by the whitefly is equivalent to a decrease in inoculations. Furthermore, acylsugars offer an advantage over many other whitefly control methods in that they are unlikely to lead to the development of resistance in the whitefly. While pesticides exert a strong selective pressure on the insect population, tomatoes are not even a preferred host of whiteflies, and whiteflies could likely identify another source of food preferable to acylsugar-covered tomato plants, thus minimizing the selective pressure on the whiteflies to become acylsugar-tolerant.

Efforts have been focused on the introgression of the genes for acylsugar production from *S. pennellii* LA 716 into cultivated tomato (Mutschler and Wintermantel, 2006). Initial lines generated in this program possessed strong insect resistance, but were

found to have a wide range of horticulturally undesirable traits due to linkage drag, including delayed germination, delayed fruit set and size, delayed maturity, and reduced seed set. It was determined that these lines carried 7 or 8 introgressions from *S. pennellii* accounting for 25-30% of the genome (Mutschler et al., 2005). Significant efforts have been invested in reducing the number and size of the introgressions from *S. pennellii* in these acylsugar tomato lines, with the most recent published report describing lines with as few as 4 introgressions from *S. pennellii* accounting for as little as 10% of the genome (Lobato-Ortiz et al., 2007). It is expected that the pyramiding of acylsugar-mediated whitefly resistance from these materials with genes for TYLCV resistance will have a serious impact on the future success of tomato cultivars in regions with significant TYLCV pressure.

Conclusion

The speed with which TYLCV has risen in prominence during the last two decades has made research on TYLCV control methods a top priority around the world. While no one method has yet proven to be a complete solution to the TYLCV problem, various combinations of physical and chemical methods for whitefly control and cultural and genetic methods for virus control have been successfully deployed in many regions with heavy TYLCV pressure. Unfortunately, all of these control methods require access to resources including physical materials, seeds, and most importantly, money, that are not always available in the developing world. TYLCV does not recognize international borders, and has become a tremendous constraint to tomato production in West Africa in the last three decades. The following section reviews the history of the development of tomato-infecting begomoviruses in West Africa and their impact on tomato production in the region.

III. *The Emergence of TYLCD in West Africa*

West Africa is defined by the United Nations as the portion of the African continent south of the Sahara desert and west of an imagined north-south axis lying at approximately 10º E longitude. The region is bordered both to the west and to the south by the Atlantic Ocean, and is situated entirely between the Tropic of Cancer and the Equator. The region has a range of climatic zones that vary both in average temperature and in the amount and timing of seasonal rainfalls. Arrayed in bands that run east-west across the continent, these zones run from the Sahel (250 – 500 mm annual rainfall) in the north through the Sudano-Sehelian Zone (500 – 900 mm) and the Sudanian Zone (900 – 1100 mm) to the Guinean Zone (>1100 mm) in the south. Countries in the region include Benin, Burkina Faso, Côte d'Ivoire, Cape Verde, The Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, and Togo. Of these 16 countries, 13 are on the United Nations list of Least Developed Countries (LDCs), with only Côte d'Ivoire, Ghana, and Nigeria remaining outside that classification.

Agriculture is the major industry of West Africa, with 60% of the regional workforce engaged in agricultural activities (FAOSTAT, 2009). In some of the least developed countries, this number climbs as high as 90%. Agriculture in this region is typically low-input, as access to resources, including credit, improved seeds, chemical inputs, farm machinery, and even extension services is severely limited (Breman et al., 2001; Kelly et al., 2003). Other significant constraints include highly degraded soils (Sanchez, 2002), shifting weather patterns (Giannini et al., 2008; Nicholson, 1980), and a wide range of damaging pests and diseases (Nwilene et al., 2008).

Tomatoes are an extremely popular vegetable in West African cuisine (see next chapter for more details) and are grown throughout the entire region. Major production areas tend to be concentrated in the drier, more northern zones, especially the Sudano-Sahelian Zone, where lower humidity limits the development of fungal diseases, which are a tremendous constraint to tomato production in the southern regions. The Sahel is characterized by a heavy rainy season of no more than four months centered on August (Giannini et al., 2008), and tomatoes are typically grown immediately following the rainy season, with wells dug to access the increased groundwater supply used as a source of irrigation. Irrigation is additionally provided by several major rivers that run through the arid regions of West Africa. The largest of these, the Niger river, brings water to arid regions of Mali, Burkina Faso, Benin, and Niger. Other rivers include the Senegal River (Mali, Senegal), the Gambia River (Gambia, Guinea, Senegal), and the Volta (Burkina Faso, Ghana).

Agricultural intensification in the West African tomato sector over the last several decades (described in detail in the next chapter) has resulted in ideal conditions for the development of epidemic TYLCD throughout the region. The arid climate combined with irrigated cropping systems is ideal for whitefly proliferation (Seal et al., 2006), which is only compounded by the prevalence of preferred whitefly hosts such as okra and cotton (Omondi et al., 2005). Widespread cultivation of a very limited set of tomato cultivars has offered tomato-infecting begomoviruses a tremendous pool of hosts in which to replicate and evolve, while limited access to new materials has left farmers with no means of halting the spread of the virus short of ceasing cultivation of tomato, and no means of improving their yields.

History of TYLCD observations in West Africa

TYLCD-like symptoms were first observed in West Africa in 1974 on tomato plants in a home garden in southern Nigeria (Lana and Wilson, 1976). Infected plants of the variety Atom Bite showed stunting and small leaflets with yellowing and upward cupping. The disease was transmissible to all other tomato varieties tested by grafting and by whitefly inoculation, but not by sap inoculation, implying a begomovirus-caused disease. However, tools for the molecular analysis of TYLCV did not exist at the time and therefore no more is known about the disease observed in that instance.

Similar disease symptoms were observed in Senegal in 1976, The Gambia in 1978, Mauritania in 1979, Côte d'Ivoire in 1980, and Mali in 1983, often with devastating results (reviewed by D'hondt and Russo, 1985). For instance, D'hondt and Russo observed some regions of Senegal with disease incidence reaching nearly 100%. They performed the first characterization of a West African tomato-infecting begomovirus to go beyond symptomology and transmission studies by describing the disease-mediated changes in subcellular structure observed by electron microscopy. These changes, which included the accumulation of virion particles and inclusion bodies, conformed with those described previously for geminivirus infection (Goodman, 1981), providing further support to the notion that the observed disease was caused by a begomovirus. Interestingly, their report describes the screening of not only 30 tomato cultivars, but also *S. pimpinellifolium* LA 121 for resistance to the virus. LA 121 had previously shown to be resistant in the Middle East (Pilowsky and Cohen, 1974). While all 30 tomato cultivars were completely susceptible, LA 121 showed only mild susceptibility, implying the possibility that the observed begomovirus was in fact a member of the TYLCV cluster or a closely related variant.

With the isolation of the TYLCV genome in 1988 (Czosnek et al., 1988) further tools began to be available for the characterization of tomato-infecting begomoviruses as they were identified around the world. A study published in 1989 documents the first use of a DNA probe to identify TYLCV in symptomatic tissue samples by squash blot hybridization (Navot et al., 1989). Squash blot hybridization involves the squashing of leaf disks on a charged nylon membrane – as cells rupture and release their contents onto the membrane, the DNA is electrostatically attracted to the membrane and remains fixed there. Such blots are extremely stable, with their diagnostic capacity remaining intact for months at room temperature, making them a very useful tool for the diagnosis of plant diseases in remote locations. Unfortunately, the nucleic acid hybridization mechanism only provides an approximate indication of the sequence similarity between the squashed sample and the probe – chimeric sequences, for instance, which are common among begomoviruses, can often go undetected unless multiple probes spanning the length of the viral genome are used.

The first worldwide survey of TYLCV using squash blot hybridization was conducted in 1990 (Czosnek et al., 1990). Samples from symptomatic plants were collected in the Mediterranean Basin (Cyprus, Egypt, Israel, Italy, Lebanon, and Turkey), West Africa (Cape Verde, Mali, Nigeria and Senegal), Southeast Asia (Taiwan, Thailand) and the Americas (Florida, Costa Rica, Venezuela) in 1987, 1988 and 1989. The DNA probe used for TYLCV detection was generated from the intergenic region of TYLCV-IL, a genomic region found in all geminiviruses that differs significantly between viral species and is associated with the regulation of gene expression and proper encapsidation of viral genomes (Lazarowitz, 1987). Samples positive for TYLCV infection were identified from all locations in the screen except for those in the New World. While still not demonstrating that TYLCV-IL was the actual viral strain

responsible for TYLCD in West Africa, this study did imply that the African, Asian and Mediterranean tomato-infecting begomoviruses are descended from a common ancestor not shared with the New World tomato-infecting begomoviruses. (Notably, the Israeli isolate of TYLCV had not yet been introduced in the New World, and thus all New World begomoviruses included in the survey were endemic to the Americas.)

In 1995 a survey of tomatoes with TYLCD-like symptoms was conducted in Burkina Faso (Konate et al., 1995). The authors described the disease as economically important in the country, and observed approximately 60% disease incidence in a high-pressure year. A serological method was used to detect the differences in antigenic properties between Burkinabé virus samples and others isolated in Senegal and Italy. The begomovirus CP gene, which codes for the coat protein that makes up the viral capsid, is highly conserved across begomovirus species since it must retain specificity for the virus's whitefly vector. Nonetheless, a panel of monoclonal antibodies raised against a series of begomoviruses can have enough differential hybridization with different begomovirus coat proteins to distinguish between some begomovirus strains (Harrison et al., 1991). In an Enzyme-Linked Immunosorbant Assay (ELISA) using a panel of monoclonal antibodies raised against *African cassava mosaic virus* the authors identified two distinct tomato-infecting begomovirus strains in Burkina Faso. One, from the Sahelian region in the far north of the country, showed a similar, though not identical, serological profile to TYLCSV (the Western Mediterranean virus), while another, from the Kou Valley in the central Sudano-Sahelian region of the country had a similar profile to an isolate from Senegal. Interestingly, the northern isolate had a very similar profile to several viral isolates from tobacco in the same region, implying the possibility that those isolates represent a single virus with a host range that includes both tomato and tobacco. Also interesting

is that isolates of Okra-infecting and tomato-infecting begomoviruses from the Kou Valley showed extremely similar serological profiles, supporting the possibility that they are adapted to transmission by the same whitefly variant. However, unlike with DNA sequences, similarities in serological profiles are not automatically assumed to imply relatedness, and therefore little can be considered validated by this study beyond the existence of two distinct tomato-infecting begomovirus variants with economic importance in Burkina Faso.

A second worldwide survey of tomato-infecting begomoviruses, based again on squash blot hybridization, was conducted in 1997 (Czosnek and Laterrot, 1997). In the seven years since their initial survey, the authors had collected additional samples from the Eastern Mediterranean Basin and the Middle East (Jordan, Syria), the Western Mediterranean Basin (Spain), Sub-Saharan Africa (Burkina Faso, Cameroon, Côte d'Ivoire, Sudan, and Tanzania), Central Asia (Turkmenistan), and the Americas (Cuba, the Dominican Republic, Argentina). Many of these new samples represented newly emergent strains of tomato-infecting begomoviruses, while those in the Dominican Republic and Cuba were known to have been introduced from the Middle East in the intervening years. All samples from the original study were also included in the new survey. Several new approaches were used in this survey to elicit further information from the squash blot hybridization results. Firstly, two DNA probes were utilized – the first, from the intergenic region of TYLCV-IL, served as a specific probe for that strain of the virus while the second, a full-length clone of the same TYLCV strain, served as a more general probe. Secondly, hybridizations were performed at two different levels of stringency to differentiate between strong and weak sequence identity. This allowed each sample in the survey to be placed into one of four categories based on the strength of its apparent relatedness to the Israeli TYLCV.

Using this protocol all samples from the Eastern Mediterranean and Middle East, as well as those from Cuba and the Dominican Republic, were given a score of 4, indicating hybridization of both the general and specific probes under high stringency conditions. In contrast, the samples from the Western Mediterranean and Southeast Asia received scores of 2, indicating hybridization of both the general and specific probes only under low stringency conditions. The samples from West Africa fell into three categories. Those from Senegal and Cape Verde received scores of 3 (hybridization of the general probe under high stringency, but hybridization of the specific probe only under weak stringency), while samples from Côte d'Ivoire, Mali and Nigeria received scores of 2, and samples from Burkina Faso received a score of 1 (hybridization of the general probe only under weak stringency), the same score received by samples from Argentina, Cameroon, Tanzania and Turkmenistan. These results imply a significant level of diversity in the tomato-infecting begomoviruses of West Africa, and further imply that some West African isolates share a more recent common ancestor with the Middle Eastern strains of the virus than is shared between begomovirus isolates from the Eastern and Western Mediterranean Basin. Whether this was indicative of a recent recombination event or a more ancient divergence in phylogeny was not revealed by the study.

As of 2002 when the first West African begomovirus genome sequence was published, the abovementioned studies were the only reports of TYLCD incidence in West Africa. They paint a picture of a diverse viral complex that was widespread throughout the region and was, at least in isolated incidences, highly destructive, but the lack of more detailed descriptions of the impact of the disease on tomato production makes it difficult to know with certainty how important the disease was in the region. Bacterial wilt, early blight, Fusarium wilt, root knot nematodes, *Helicoverpa armigera*, and

spider mites are also major pests and diseases of tomato in West Africa, and few reports were published on those constraints as well. However, given the existence of a diverse set of tomato-infecting begomoviruses throughout West Africa going back to at least the mid-1990s, and given that the tomato-infecting begomoviruses of West Africa have more recently been characterized to be a major constraint to production (as described below), it seems reasonable to conclude that by the mid-1990s TYLCD was, at the very least, one of the region's more damaging diseases of tomato.

The Tomato-Infecting Begomoviruses of West Africa

The detailed characterization of West African tomato-infecting begomoviruses began with the sequencing of the genome of a West African TYLCD-associated begomovirus in 2002 (Théra et al., 2002). Isolated in Mali, this begomovirus was found to most closely match a Middle Eastern TYLCV isolate in most of its sequence, but it most closely matched *Hollyhock leaf curl virus* (HLCrV) from Egypt in its C1 and C4 ORFs. At the time it was tentatively described as an isolate of TYLCV, and therefore dubbed TYLCV-Mali. Since then, it has been identified as a separate begomovirus species *Tomato yellow leaf curl Mali virus* (TYLCMLV, Fauquet and Stanley, 2005), and further isolates have been identified in Ethiopia (Shih et al., 2006), and Ghana (Osei et al., 2008).

With the advent of the Agricultural Biotechnology Support Project II (ABPSII) program on tomato in West Africa in the last several years (described in detail in the next chapter), significant work has been done by the Robert Gilbertson lab at U.C. Davis to fully characterize the tomato-infecting begomoviruses of West Africa. Samples have been collected in Benin, Burkina Faso, Ghana, Mali, Niger, Nigeria, Senegal, and Togo, and viral genomes from all locations have been amplified and

sequenced. Descriptions of a total of three West African tomato-infecting begomovirus species, one associated with a DNA-β satellite, have been published. They are *Tomato yellow leaf curl Mali virus* (TYLCMLV), *Tomato leaf curl Mali Virus* (ToLCMLV), and *Tomato yellow leaf crumple virus* (ToYLCrV).

TYLCMLV causes typical TYLCD-type symptoms in infected plants, including stunting, leaf yellowing and cupping, flower abscission, and significant yield losses when plants are infected early. It has been identified thus far in Benin, Burkina Faso, Ghana, Mali, Senegal, and Togo, often causing up to 100% disease incidence in those countries during peak whitefly season. As described earlier, it is a recombinant virus in which the majority of the genome is derived from a Middle Eastern TYLCV isolate known as TYLCV-Mld, while the remainder is most closely related to HoLCrV (Chen et al., 2009). Importantly, sequence similarity between TYLCMLV and these two viruses is approximately 90%, implying a period of geographic isolation during which mutations accumulated in the TYLCMLV genome following recombination. In other words, TYLCMLV is not a recent recombinant, though the exact timing of the recombination event is difficult to calculate. This is further supported by the existence of the Ghanaian and Ethiopian strains of TYLCMLV, which are 97 and 91% identical to TYLCMLV, respectively – for the Ethiopian strain to have diverged 9% from the Malian strain implies that significant time has elapsed since the two shared a common recombinant ancestor. TYLCMLV does fall within the TYLCV complex of tomato-infecting begomoviruses, but it falls within a sister clade to the Middle Eastern TYLCV strains and is in fact its own species.

TYLCMLV has been found to be associated with a DNA-β satellite that is not required for the virus's replication but which can significantly increase the symptom

severity of a TYLCMLV infection (Chen et al., 2009). Plants co-infected by TYLCMLV and its DNA-β show a severe stunting sometimes described as a "broccoli" symptom. Sequence analysis of this satellite molecule has shown it to be nearly identical to a DNA-β known as *Cotton leaf curl Gezira betasatellite* (CLCuGB), a satellite typically associated with malvaceous species such as cotton and okra that has been found in begomovirus-infected okra in Sudan and Mali. Given the promiscuity of many DNA-βs and the polyphagous nature of the B-biotype of *Bemisia tabaci*, which in Ghana has been shown to favor both okra and tomato as hosts (Omondi et al., 2005), it is perhaps not surprising that a tomato-infecting begomovirus has been found associated with a DNA-β more commonly found in okra. Since a minor portion of the TYLCMLV genome is descended from a malvaceous begomovirus (an ancestor of HoLCrV), it has been proposed that the recombination event gave a selective advantage to TYLCMLV by allowing it to associate with a common African DNA-β satellite, perhaps increasing its host range or its replication capacity (Chen et al., 2009). While this theory is intriguing, the majority of TYLCMLV infections do not involve the DNA-β, and no specific advantage has been found to co-infection by the virus and the DNA-β.

ToLCMLV and ToYLCrV both cause the common TYLCD-associated symptoms in tomato with minor variations: ToLCMLV does not cause significant leaf yellowing, and instead often causes a purple veination pattern, while ToYLCrV does cause yellowing but causes leaves to crumple rather than curl. Analysis of their genome sequences shows that both viruses are closely related to each other, but fall outside the major TYLCV complex (Zhou et al., 2008). In fact, ToLCMLV and ToYLCrV fall into a clade of African begomoviruses, and their next closest known relatives are

Tobacco leaf curl Zimbabwe virus and *Tomato curly stunt virus* from South Africa. Both can be found in Benin, Burkina Faso, Ghana, Mali, Niger, Senegal, and Togo.

Importantly, mixed infections of two or more of these begomoviruses have been observed in single tomato plants throughout West Africa. When ToYLCrV is involved, multiple symptom types are visible on the same plant. The results of mixed infections are typically stronger disease symptoms – in moderately infected fields, plants with mixed infections stand out due to their stronger than average stunting.

In addition to TYLCMLV, ToYLCrV, and ToLCMLV, two additional West African tomato-infecting begomoviruses have been identified through the ABSPII West African tomato program (Gilbertson RL, personal communication). One was isolated in Nigeria, and is provisionally named *Tomato leaf curl Nigeria virus* (ToLCNgV) and the other was isolated in Togo and is provisionally named *Tomato leaf curl Togo virus* (ToLCTgV). Full genome sequences place the two viruses within the African begomovirus clade. A DNA-β satellite has been found in association with ToLCTgV; while it does cluster with the African DNA-β sequences, it is different from previously identified satellites and is still being characterized.

Conclusion

The diversity of tomato-infecting begomoviruses in West Africa, including viruses both from within and without the TYLCV cluster, implies the existence of a significant pool of genetic variation enabling the viruses to adapt to new hosts as they become prevalent in the region. In addition, the regional environment has several features such as high temperatures, frequent irrigation in arid areas, and a prevalence of whitefly-favored hosts such as okra and cotton that enable the accumulation of

significant whitefly populations in the region. The result is that West Africa is highly susceptible to the emergence of new begomovirus diseases as crop production is intensified. Given the additional regional constraints including a wide variety of damaging pests and diseases, unpredictable weather patterns, degraded soils, and lack of access to credit, inputs and markets, it is clear that the potential for emergence of new, highly damaging begomoviruses in the region is a significant threat to an already fragile system.

The following chapter describes the history of tomato production in West Africa, from its colonial roots to its significant intensification and collapse in the latter decades of the 20th century. It then moves on to discuss projects that have been underway in West Africa in recent years to alleviate the impact of tomato-infecting begomoviruses in the region and to improve access to seeds of modern tomato cultivars.

CHAPTER 2

TOMATOES IN WEST AFRICA: GROWTH, DECLINE, AND PLANS FOR RECOVERY

Introduction and Intensification

While not indigenous to West Africa, tomatoes have been a part of the region's culinary landscape for hundreds of years. Originally domesticated in the New World (Bai and Lindhout, 2007), tomatoes were likely brought to Africa by European explorers or merchants in the 16th century. The history of their introduction and subsequent integration into the local cuisine are unfortunately not well documented due to a general lack of recorded histories among African peoples, but primary literature from outside traders, colonialists and explorers implies that tomatoes have likely been part of the West African diet since at least the mid-19th century. An 1849 botanical treatise on the flora of West Africa, for instance, describes tomato as being commonly cultivated in Fernando Pó, an island in the Gulf of Guinea which was, at the time, an administrative center of the British Empire (Hooker et al., 1849). The treatise additionally describes tomato as being commonly found escaped from cultivation throughout Africa, supporting the notion that tomatoes were readily available for culinary use in West Africa at that time. In 1863, a travelogue titled "Wanderings in West Africa from Liverpool to Fernando Po" describes a meal in Accra (now the capital of Ghana) in which tomatoes were an ingredient in "Palaver sauce" (Burton, 1863), a mixed-vegetable stew that is still today a staple of West African cuisine, typically made with greens, tomatoes and meat and served over a starch such as rice, yams, or a porridge known as fufu. While this meal was served by a British colonialist, it consisted of local specialties and was prepared by local people, implying that tomato likely was a locally-used ingredient by that time. By 1898 there

is evidence that tomatoes were intentionally cultivated by people thoughout West Africa. A lengthy treatise on the British territories in Africa published in that year describing the land and its flora and fauna, as well as its inhabitants and their customs, mentions “degenerate” tomatoes growing “semi-wild” around most villages in the region (Johnston, 1898). While the author of that treatise may not have had the highest regard for the locally favored tomato varieties in West Africa at that time (tomatoes had undergone a second round of intense domestication in Europe in the 18th and 19th centuries, Bai and Lindhout, 2007) his observation of tomatoes surrounding most villages strongly supports the notion that people were eating the tomatoes, and either intentionally cultivating them or at least allowing them to grow as volunteer plants from dropped tomato seeds. By the 1920s, cookbooks for West African colonial administrators were making specific references to buying tomatoes in local markets, demonstrating that by that time tomatoes were undoubtedly being grown for consumption in the region (Tew, 1920).

While the exact timeline of the integration of tomatoes into the West African culinary canon is not easily discerned, it is clear that by the 1960s, when most West African nations were gaining their independence, tomatoes had become integral to the regional diet. In what is now considered classic West African cuisine, tomatoes are typically fried in oil with onions and made into a paste that then serves as a base for many different stews and sauces. As a result, when commercial canned tomato paste became available in West Africa, it quickly increased in popularity, especially in the growing urban centers. In 1967, for instance, West Africa imported 18,000 tons of tomato paste from Italy alone (NAS, 1974).

Recognizing a significant opportunity, farmers in West Africa began increasing their tomato production starting in the 1960s, with land under tomato cultivation more than doubling in the decade between 1961 to 1971, and doubling again from 1971 to 1981 (FAOSTAT, 2009). Several young West African governments saw domestic tomato production and processing as a major opportunity for economic development, and invested heavily in infrastructures to support the growing industry. In 1964, for instance, a state-owned cannery was opened in Mali in the town of Baguineda, a major irrigated production area outside the capital city of Bamako (Kelly et al., 2005). Like many other areas scattered throughout Francophone West Africa, Baguineda benefitted from a pre-existing irrigation project built decades earlier by the French colonial service (Eicher and Baker, 1992), and was therefore an ideal location for high-yield tomato production. Built in part with funds from the Yugoslavian government, the cannery had two processing lines, one for tomato paste and the other for mango puree. Both were intended to help provide a market for surplus horticultural production during the peak harvest season, and to decrease dependence on canned imports by providing a domestic alternative. The country began a major campaign to encourage farmers to grow tomatoes – many of the older farmers throughout Mali today recall that campaign as the primary impetus for their adoption of tomato cultivation at the time (Soumaré and Moore, 2005).

A similar project was initiated in Ghana starting in 1968 (Khor and Hormeku, 2006). Three state-owned tomato processing facilities were opened that year in three geographically distinct locations throughout Ghana: one in the northern region in the town of Pwalugu, one in the central region in the district of Wenchi, and one in the south in Nsawam, near Accra. The canneries operated on partial contract arrangements with smallholder growers, guaranteeing to buy specific quantities of tomatoes at set

prices, which had the added value of providing farmers with information about market pricing and thus giving them greater control when negotiating with buyers for fresh market distribution. In 1975, Ghana additionally initiated a major irrigation project in the Upper East Region to develop an area of 24,000 hectares for the growing of irrigated crops. The Tono dam was completed in 1985 and is one of the largest agricultural dams in West Africa. Since its completion, close to 90% of the two million people living in the Upper East Region have taken up tomato growing, finding tomato to be more lucrative than all other crops (Khor and Hormeku, 2006).

These and other tomato-related projects sponsored by governments, including research and extension programs in many countries (NAS, 1974), led to significant increases in tomato yields throughout West Africa in the 1960s, 70s and 80s. In the 1960s tomato yields in West Africa averaged just 6.3 tons per hectare (t/ha), but starting in the early 1970s they began to rise steadily and by 1990 had reached 13.0 t/ha (FAOSTAT, 2009). However, it is worth noting that in contrast, tomato yields in the United States were 25.4 t/ha in 1961 and had reached 55.1 t/ha in 1990. Thus, while African governments were encouraging significant agricultural intensification around tomato production, major constraints were still preventing farmers from producing at the levels seen in the developed world.

Policy Changes and Decline of the Tomato Processing Industry

In the mid-1980s the International Monetary Fund (IMF) and the World Bank began changing their policies for lending to developing countries. Based on the principle that loans to a country with significant debt should be accompanied by a series of conditionalities that move that country towards greater liquidity of resources and thus increased capacity to cover its expenses, the IMF and the World Bank began requiring

loan recipients to institute a series of Structural Adjustment Programs (SAPs, Williamson, 1983). These SAPs were very broadly designed to allow free market forces, rather than government policies, to set industry and trade priorities. A series of measures such as the lifting of import and export barriers, the removal of price controls and state subsidies, and the privatization of state-owned enterprises were enacted across West Africa in the late 1980s and early 90s.

The effects of the SAPs on tomato processing in West Africa were felt quickly and decisively. Countries sold their canneries to private buyers, who soon found that they could not compete against the subsidized tomato paste imported from Europe (Sumner et al., 2001) that began flooding the market when import restrictions were lifted. Tomato yields in West Africa were simply too volatile for consistent tomato paste production, and consistent production was necessary for profitability in the face of such stiff competition. (In contrast, when the canneries had been government owned they could operate at a loss if necessary for greater social welfare.) By the mid-1990s, two of the three canneries in Ghana had closed (Khor and Hormeku, 2006), as had the cannery in Mali (Kelly et al., 2005), leaving tomato farmers without the extension services, markets, and bargaining power they had come to depend upon.

Since that time, tomato production in West Africa has been in a state of flux. A trend towards urbanization (UNOWA, 2007) has created strong localized demand for both canned and fresh tomatoes in major cities. Tomato paste imports from Europe and, more recently, from China, have skyrocketed, from approximately 29,000 tons in 1990 to 222,000 tons in 2006 (FAOSTAT, 2009). Fresh market demand has also increased, and while tomato producers in many of the most concentrated tomato-producing regions have had success marketing their yield to the growing urban population, their

situation is fragile and easily disrupted by a single misstep. While production levels have increased with increasing land under cultivation, yields have been unpredictable due to a wide variety of issues including TYLCD and other diseases, lack of finance, inconsistent seed quality, lack of access to chemical inputs, and unpredictable weather (Adu-Dapaah and Oppong-Konadu, 2002; Asare-Bediako et al., 2007; Maatman et al., 2004; Soumaré and Moore, 2005). Farmers often find themselves with insufficient yields to make a decent profit, and when yields are high there is often a short-term glut on the market driving prices down practically to zero and making it difficult for farmers to find buyers. Furthermore, a very small number of players control the produce markets in some of the major urban areas throughout West Africa, giving farmers little bargaining power: if they don't accept the buyers' price offers, the buyers simply take their trucks elsewhere, often across the border into neighboring countries to find a better price (Kufuor, 2008).

Projects for the Improvement of Tomato Production in West Africa

The intensification of tomato production in West Africa followed by the decline of the tomato processing industry has made tomato the highest priority vegetable crop for improvement in the region, according to a consortium of West African government agricultural researchers (Levasseur, 2004). The sheer number of constraints on tomato production makes it difficult to even know where to begin recovery efforts, but TYLCD has become a particular focus for several reasons: it is a recently developed and highly pervasive problem, its levels fluctuate from year to year, making predictions of its impact difficult, and significant worldwide efforts have been devoted to its control, offering many options for programs to alleviate the impact of the disease in West Africa. This section describes a series of projects started in 1998 for the

control of tomato-infecting begomoviruses and the improvement of tomato production in West Africa.

Integrated Pest Management Collaborative Research and Support Program

Recovery efforts focused on TYLCD began with the initiation of a project in Baguineda, Mali in 1998 to characterize the nature of West African TYLCD-causing viruses and to develop a strategy for their control. The loss of the tomato cannery had been devastating to the farming community in Baguineda, and was blamed primarily on increased incidence of whitefly-vectored diseases that had grown in prevalence in the region since the 1980s, leading many farmers to quit tomato production for other crops (Noussourou et al., 2008; Soumaré and Moore, 2005). Funded by the United States Agency for International Development (USAID) and coordinated by the Integrated Pest Management Collaborative Research Support Program (IPM CRSP), the project in Baguineda identified TYLCMV as the primary disease agent of TYLCD in the region (Théra et al., 2002) and evaluated a number of potential cultural approaches to management of the virus (IPM-CRSP, 2001). Given the existing organization of farmers in Baguineda, which had arisen due to the management necessities of the local irrigation system, the implementation of a three-month host-free period preceding the primary tomato growing season was determined to be the most optimal approach for TYLCD control. With the help of local extension officers, a host-free period excluding both tomatoes and peppers from cultivation during June, July and August was implemented starting in 2004 (Noussourou et al., 2008). While compliance in the first year of the project was limited due to skepticism on the part of the farmers, incremental yearly improvements convinced growers that the host-free period could have a significant impact on the incidence of TYLCD, and compliance

did increase dramatically over time, such that by 2006 only .005 hectares of tomatoes or peppers could be found growing during the host-free period in all of Baguineda.

Unfortunately, despite the significant decreases in TYLCD incidence precipitated by the adoption of the host-free period, yields in Baguineda did not increase as dramatically as expected. Access to modern cultivars in West Africa was severely limited, and many of the varieties in use in the region were several decades old. The IPM CRSP project introduced several modern tomato hybrids in Baguineda to assess their performance under the host-free period conditions. Despite having no resistance to TYLCD, these varieties significantly outperformed the popular local cultivars under the reduced viral load following the host-free period (Noussourou et al., 2008). It was determined as a result that future work in the region should focus on the introduction of modern high-yielding tomato varieties.

Agricultural Biotechnology Support Project II

As the IPM CRSP project began the implementation of a host-free period in Baguineda in 2004, AVRDC-The World Vegetable Center established a satellite center in West Africa and began to explore the ways in which vegetable production could be improved in the region. A workshop of government agricultural researchers from many West African nations was convened at the AVRDC regional headquarters in Bamako, Mali in March of 2004 to set priorities and plan research activities (Levasseur, 2004). By consensus, tomato was selected as the highest priority crop due to its economic importance in the region and the wide range of constraints limiting its production.

In 2005, as IPM CRSP was winding down its involvement in Baguineda, a new USAID-funded project was getting started. The Agricultural Biotechnology Support Project II (ABSPII) aims to improve agricultural production in the developing world through biotechnology, and initiated a project in 2005 to address tomato production in West Africa. A partnership was formed between researchers at AVRDC, Cornell University, and the University of California-Davis (UC Davis), the latter having been partners on the IPM CRSP project in Baguineda. National agricultural research services (NARS) of seven West African countries – Benin, Burkina Faso, Ghana, Mali, Niger, Senegal, and Togo (Table 2.1) – joined up with the management team to address the tomato begomovirus epidemics in West Africa and to improve the quality of germplasm available in those countries. A multi-pronged approach was laid out, including molecular biology work to characterize the tomato-infecting begomoviruses of West Africa, breeding work to combine potyvirus and begomovirus resistances in a single tomato variety, and a series of variety trials to identify germplasm well-adapted to the region, with significant capacity-building components to ensure that the work

Table 2.1 – National agricultural research services (NARS) of the seven West African countries participating in the vegetable germplasm trialing network

Country	Institution	Abbreviation	Location
Benin	Institut National de Recherche Agricoles	INRAB	Cotonou, Bénin
Burkina Faso	Institut d'Etudes Environnementales et de Recherches Agricoles	INERA	Bobo Dioulasso, Burkina Faso
Ghana	Crop Research Institute	CRI	Kumasi, Ghana
Mali	Institut d'Economie Rurale	IER	Bamako, Mali
Niger	Institut National de Recherche Agronomique du Niger	INRAN	Niamey, Niger
Senegal	Centre pour le Developpement de l'Horticulture/Institut Senegalais de Recherche Agricole	CDH/ISRA	Dakar, Senegal
Togo	Institut Togolais de Recherche Agricole	ITRA	Lomé, Togo

could continue in West Africa after the project ended. In addition the project was designed to interface with any establishments associated with seed increase and distribution that might develop in West Africa during the duration of the project to help establish a pipeline of varieties from the trials to farmers' fields.

Characterization of West African Tomato-Infecting Begomoviruses

As described in the previous chapter, a significant component of the ABSPII project was the identification of the tomato-infecting begomoviruses responsible for TYLCD in West Africa. Led by the Gilbertson lab at U.C.Davis, this work involved the collection of thousands of begomovirus samples from potentially infected plants identified throughout West Africa. Leaf discs of crop plants and weeds showing disease symptoms were squashed onto nylon membranes in Africa, and the membranes were then analyzed by either squash blot analysis or PCR to detect begomoviruses and to determine their identities. Though this work was initially done entirely by visiting scientists from U.C. Davis during twice-yearly trips to West Africa, the sample collection was later performed by researchers from the West African NARS as well. This allowed for the collection of samples from a wider geographic distribution, as well as the training of NARS researchers in the basics of DNA sample collection in the field. From this research at least five new tomato-infecting begomoviruses were identified, as discussed in chapter 1 of this work.

To further build biotechnology capacity in West Africa, a weeklong intensive molecular biology training workshop was held at the University of Bamako in August 2007 to teach the NARS partners basic molecular biological theory and practice through integrated lectures and laboratory exercises. Over 20 participants from all seven partner countries learned a wide range of relevant topics including basic

molecular biology and genetics; techniques for detecting, manipulating and identifying nucleic acids; genetic engineering and plant transformation; basic molecular plant pathology and disease detection; and protein detection and analysis. In the laboratory, participants performed squash blot hybridizations to identify begomoviruses in local tomato samples, conducted PCR amplification to genotype transformed and untransformed plant DNA, and conducted an ELISA to test for bacterial wilt in eggplant. Whereas most of the partner countries' agricultural research institutions lack the necessary equipment and supplies for these methods, the knowledge-building associated with the workshop was designed to enable the project's research partners to make informed decisions when establishing molecular research programs in the future. In order to provide an immediate practical benefit from the workshop, however, participants were also trained in the use of kits with minimal equipment requirements, such as ImmunoStrips (Agdia Inc., Elkhart, IN), which allow diagnostic tests for disease identification to be performed in the field.

Establishment of a Regional Vegetable Germplasm Trialing Network

The primary focus of the ABSPII project in West Africa was the establishment of a region-wide vegetable germplasm trialing network that could independently evaluate new varieties from around the world for adaptation to local growing conditions, select, with input from farmers, the optimal varieties for the region, and move those new materials into farmers' fields in a timely manner. Over the course of three years from 2005 through 2008, this network was slowly developed through the trialing of nearly 100 putatively TYLCD-resistant tomato varieties in the seven participating countries. These materials came from a wide range of multinational seed companies and public breeding institutions (Table 2.2)**.**

Table 2.2 – Public and private seed sources providing TYLCD-resistant tomato cultivars for inclusion in the West African tomato variety trials 2005-2008

Organization	Location
AVRDC - The World Vegetable Center	Shanhua, Taiwan
CIRAD - French Agricultural Research Centre for International Development	Guadeloupe
De Ruiter Seeds	Bergschenhoek, The Netherlands
Enza Zaden	Enkhuizen, The Netherlands
Gentropic - Semillas Tropicales	Sacatepéquez, Guatemala
Harris Moran Seed Company	Modesto, CA, USA
Hazera Genetics	Shikmim, Israel
Hebrew University of Jerusalem	Jerusalem, Israel
Nunhems	Haelen, The Netherlands
Seminis	St. Louis, MO, USA
Soli	Kiryat Malachi, Israel
Syngenta AG	Basel, Switzerland
Takii Seed	Kyoto, Japan
Tropicasem	Dakar, Senegal
University of Florida	Gainsville, FL, USA
Agricultural Research Organization of Israel - Volcani Center	Bet Dagan Israel

The first year of the project (the 2005-2006 growing season) saw the systematic evaluation of 40 begomovirus-resistant tomato varieties in each of the participating countries. Designated as preliminary screening trials by the partners, these evaluations consisted of unreplicated trials, in which 26 plants of each variety were planted on agricultural research stations and evaluated for disease resistance according to a TYLCD symptom severity scale (Lapidot and Friedmann, 2002). The regionally popular variety Roma VF was used as a susceptible check, and natural levels of virus inoculum, delivered via indigenous populations of whiteflies, were relied upon for disease development in the field. Partners encountered a range of problems in the first

year, including insufficient disease pressure, poor seed germination, and inconsistent trial management practices between countries. Despite these issues, sufficient data were generated to select 11 promising varieties showing high levels of TYLCD resistance for inclusion in the next years' trials. (See chapter 3 for trial results.)

In the project's second year, the 11 varieties selected in the first year were evaluated in advanced trials. Having learned from the mistakes of the previous year, the project partners developed a detailed trialing protocol to resolve inconsistencies among locations, and to ensure the statistical relevance of the data. Trials were maintained as if for commercial production, with pesticide applications being used to control diseases and pests other than whiteflies. In addition, attempts were made to plant the trials in areas known to be free of other important regional pathogens, such as root-knot nematode, the fungus *Fusarium oxysporum* f. sp. *lycopersici* (causal agent of fusarium wilt), and the bacterium *Ralstonia solanacearum* (causal agent of bacterial wilt). Plants were evaluated for TYLCD resistance at flowering, fruiting, and first harvest using the standardized symptom severity scale. In addition, three randomly selected plants from each plot were evaluated for yield, which was extrapolated to tons per hectare.

Overall, the results of the second year's trials showed a marked improvement in quality over the previous year, indicating the value of hands-on experience in the development of germplasm trialing capacity. With few exceptions, the trials showed significant improvements in yield over typical tomato yields in the region. In several locations, trial coordinators solicited anecdotal farmer opinions on the trial materials. In these cases, farmers were generally very enthusiastic about the new varieties and provided helpful insight into their variety selection process, typically showing a

preference for medium-sized firm fruits that stand up well to shipping. At the end of the season, the top four varieties based on disease resistance and yield were selected for a third round of trials. Representatives from each country also selected two additional varieties based on local performance and preferences. (See chapter 4 for trial results.)

In addition to the 11 varieties evaluated in the advanced trials, 28 new varieties were trialed in preliminary screening trials during the 2006-2007 growing season. These preliminary screens were conducted similarly to the advanced trials, but without replication. Of the more than 60 new varieties tested, many showed significant levels of TYLCD resistance, and 20 were selected by at least one of the participating countries for inclusion in advanced trials during the project's third year. (See chapter 6 for trial results.)

The 2007-2008 growing season was the final year of the project. Top materials from the 2006-2007 trials were evaluated in multi-location trials throughout West Africa. These trials used a protocol similar to that of the advanced trials of the previous season, but were conducted on farms (as opposed to agricultural research stations) in two locations in each of the seven participating countries, preferably in different agroecological zones. While providing yet another opportunity for the evaluation of new varieties, these multi-location trials also met two important goals not addressed by the previous years' trials. Firstly, they offered an opportunity to evaluate germplasm within the context of an actual vegetable production area, rather than in the more controlled environment of an agricultural research station. Trials were managed according to the customs of the farmers in the region and, thus, offered a more realistic view of the potential performance of the selected varieties in a true production setting.

Secondly, these multi-location trials served as the bridge between germplasm evaluation and variety distribution. Situated among the fields of commercial tomato producers, these trials functioned as demonstration plots for the new cultivars, helping to spread the word about modern varieties and diminishing some of the risk associated with adoption a new, unknown technology. By the end of this growing season local farmers had seen the potential of the new varieties, and many expressed interest in conducting small on-farm trials of the selected materials. (See chapter 5 for trial results.)

A set of advanced trials using the material selected during the preliminary trials of the 2006-2007 season were also conducted. (See chapter 7 for results.) No preliminary screens were conducted during the 2007-2008 growing season as the focus shifted from evaluating germplasm to mobilizing high-performing materials into the seed distribution pipeline.

The following chapters report the results of each of the five trials conducted throughout West Africa in 2005-2008[2].

[2] The variety trials described in this document were a collaborative effort by many individuals, with separate national teams of researchers holding the responsibility for conducting the trials in each participating country. Management of the trials was divided between Cornell University and AVRDC. As the project leader at Cornell, the author held primary responsibility for all matters related to the trial's germplasm collection, including acquisition of materials, analysis of trial data for the assessment of variety performance, oversight of the variety selection process, and detailed yearly reporting to each of the project's seed donors. The author additionally performed a significant coordinatory role in the execution of the variety trials, participating in yearly planning meetings in Bamako, Mali and contributing to the design of each year's trial protocol. The author visited each of the trial sites during the 2006-2007 and 2007-2008 seasons (with the exception of the one site in Senegal in 2006-2007 and the two sites in Niger in 2007-2008). During these visits, the author provided guidance to the trial managers regarding disease scoring, yield data collection, and general trial maintenance, and additionally visited nearby farming areas to discuss the impact and management of TYLCD.

CHAPTER 3

YEAR 1: PRELIMINARY SCREENING TRIAL

Introduction

The first round of variety trials for the identification of TYLCD-resistant tomato cultivars highly adapted to the growing conditions of West Africa was conducted during the 2005-2006 growing season in eleven locations throughout West Africa. As many of the research partners managing the trials were new to variety trialing and had limited training, the trial protocol was designed to be easily executed. Forty tomato cultivars from thirteen seed sources, including commercial, public and academic organizations, were evaluated in the trial.

Materials and Methods

Plant Materials

40 tomato varieties with putative TYLCD resistance were selected by 13 seed companies and public breeding institutions for inclusion in the 2005-2006 preliminary screening trial. These varieties carry TYLCD resistance from a range of sources, including *S. chilense, S. peruvianum, S. habrochaites and S. pimpinellifolium*, and in some cases include genes pyramided from more than one source. Aside from their shared TYLCD resistance, the initial trial materials were not selected for any other common traits. Table 3.1 lists all included varieties along with the organization that provided them and, when known, their resistance sources. Seeds of two varieties, FTC 6231 from Harris Moran Seed Company, and Sasya 0202 F1 from Seminis-India, were in limited supply and therefore each was only trialed in half of the trial locations throughout West Africa.

Table 3.1 – Tomato varieties included in the 2005-2006 TYLCD resistance trials. Varieties marked with ** were only included in half the trials.

Seed Source	Variety Name	Resistance Source (where known)
AVRDC	CLN 2123A	*Ty-2*
	CLN 2460E	*Ty-2*
	CLN 2468A	*Ty-2*
	CLN 2498E	*Ty-2*
	CLN 2545A	*Ty-2*
	CLN 2545B	*Ty-2*
	PT 4722A	*Ty-2*
	TLCV 15	*Ty-2*
Cirad Guadeloupe	O4 108	
	O4 240	
	O4 495	
	O4 498	
	O4 501	
De Ruiter Seeds	Bybal	
	Industry DR 10403	
	Lety F1	
	Realeza	
	Thoriya	
Enza Zaden	Atak	
	Chenoa	
	Ponchita	
	Yosra	
Harris Moran	FTC 6231**	*Ty-1*
	FTC 6236	*Ty-1*
	FTC 7088	*S. chilense* LA 1969, *S. habrochaites* H24
	FTC 7127	*Ty-2* , *S. habrochaites* H24
	FTC 7351	*S. chilense* LA 1969 and LA2779
	FTC 7483	*S. pimpinellifolium*
	HMX 4810	*S. chilense* LA 1969
Hazera	HA 3060	
Hebrew University	Favi 9	Ih902
Seminis	GemPride	*Ty-1*
	PS 43316	
Seminis - India	Sasya 0202 F1**	
Syngenta	Cheyenne E448	
	Nirouz TH 99806	
	Yassamen TH 99802	
Takii	TY 75	*Ty-2*
Tropicasem	F1 3019 Galina	
	Nadira	
	Roma VF	Susceptible check

The variety Roma VF was used as the susceptible check in all trials. Roma VF is a very popular cultivar throughout West Africa, despite its marked susceptibility to TYLCD, and is readily available in seed markets in major urban centers throughout the region. The Roma VF seeds used in the trials were obtained from Tropicasem.

A total of 25 additional cultivars were included in select trials. The majority were additional putatively TYLCD-resistant cultivars which were available only in limited supply. These were trialed primarily by AVRDC at the Samanko research station. Performance data for these varieties are available in Appendix 2, but will not be discussed at length in this chapter. Additionally, some trials included locally popular TYLCD-susceptible varieties as additional susceptible controls – in those cases, variety performance will be mentioned alongside Roma VF performance.

The majority of the materials included in the trial were F1 hybrid cultivars. Open pollinated tomato varieties are uncommon in commercial growing settings, and since the majority of varieties were donated by commercial seed companies they were primarily F1 hybrids. The exception was the breeding lines from AVRDC.

Seeds were collected in bulk at the AVRDC regional headquarters in Bamako, Mali, where they were repackaged into trial-sized packets and distributed to the research partners located throughout West Africa. Contingent upon availability, approximately 65 seeds per cultivar were sent to each trial location.

Trial Locations

Trials were located in eleven locations in seven countries throughout West Africa. Participating agencies were INRAB (Benin), INERA (Burkina Faso), CRI (Ghana),

IER (Mali), INRAN (Niger), CDH/ISRA (Senegal), and ITRA (Togo), as well as AVRDC. Each agency chose one site for the establishment of a trial, except for IER, which as a partner on the management of the project chose four sites for trials. In all cases, trials were conducted on agricultural research stations. Efforts were made to situate trials near tomato-growing regions. Figure 3.1 shows the locations of all eleven preliminary trials conducted during the 2005-2006 growing season.

Trial Establishment and Management

Many aspects of the trial management were left to the discretion of the trial managers, who were encouraged simply to follow local customs when planting and maintaining their trials. This approach was chosen for two reasons. Firstly, it ensured that the trials would expose the cultivars under evaluation to realistic field conditions. Field management practices vary from one location to the next within West Africa, due both to differential availability of resources and to cultural conventions, and though improving West African farmers' field management techniques would be a worthwhile goal, it was important that the trials select varieties appropriate for contemporary, and not idealized, field conditions. In addition, many of the trial managers participating in this project had never run replicated variety trials before, and it was therefore decided to start with very straightforward trialing approaches and to slowly add more complex elements, such as replication and more detailed observations, over the course of the three years of the project.

Seeds were planted in seedling nurseries managed as per local customs. After three to four weeks, seedlings were transplanted into 1.5 × 6.0 meter elementary plots, one plot per cultivar, with 26 plants per plot. Within each elementary plot plants were arrayed in two rows with a spacing of .6 meters between rows, and .5 meters within rows. The

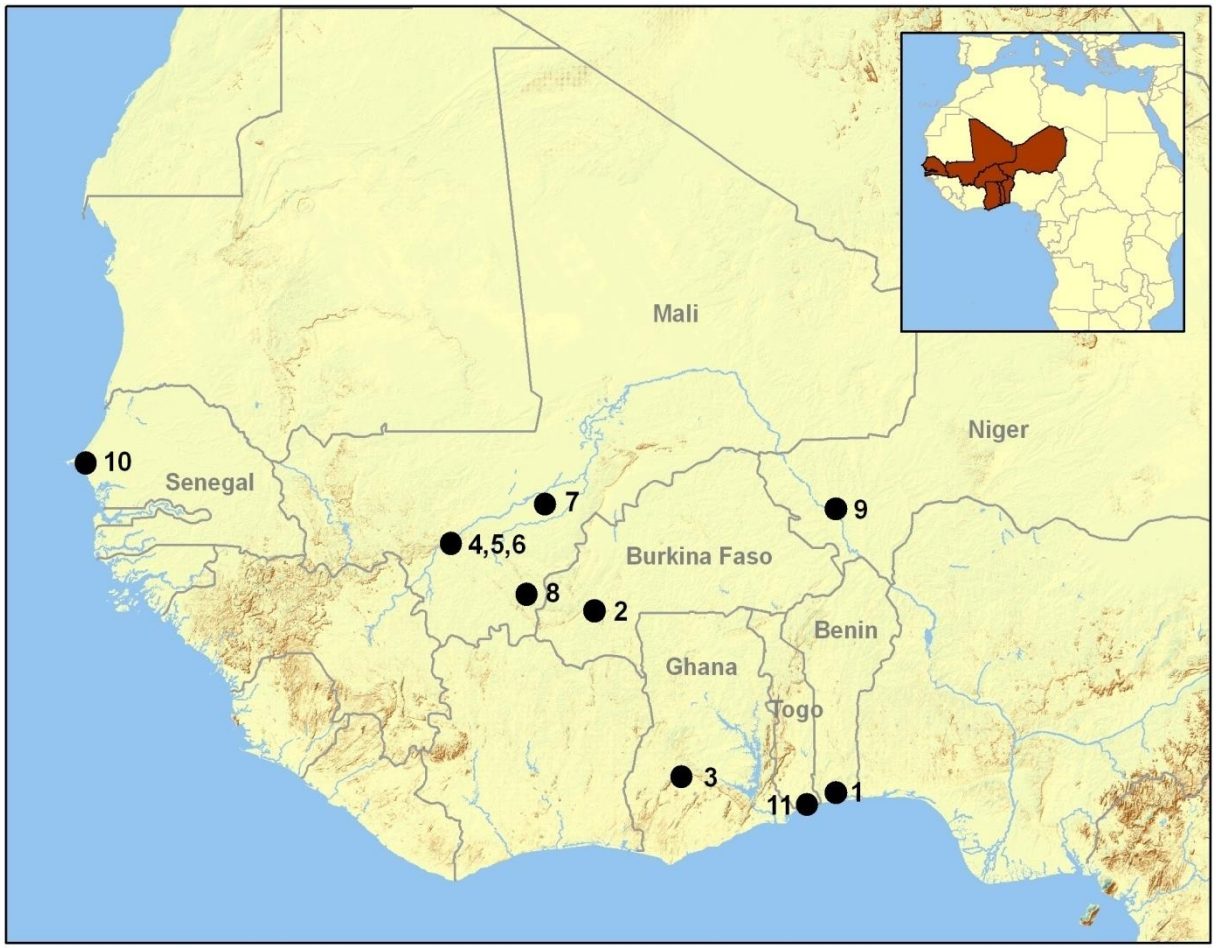

Figure 3.1 – Map of the 2005-2006 preliminary trial locations. 1 – Cotonou, Benin; 2 – Bobo Dioulassou, Burkina Faso, 3 – Kumasi, Ghana, 4, 5, and 6 – Baguineda, Samanko and Sotuba, Mali; 7 – Cinzana, Mali; 8 – Sikasso, Mali; 9 – Niamey, Niger; 10 – Rufisque, Senegal; 11 – Lome, Togo.

total trial area was 35.5 × 23 m, creating a grid of 16 × 3 elementary plots with .5 m spacing. Every fifth elementary plot was planted with the susceptible check Roma VF, and in addition the entire trial was ringed with rows of Roma VF. These large quantities of the susceptible check variety were intended to help increase the whitefly population and the pool of viral inoculum within the trial.

Plots were irrigated and fertilized in a manner typical for their locations, and trial partners were free to manage pests in any way that would not diminish whitefly populations.

Disease severity scoring and yield calculations

Scoring for disease severity was performed two weeks after transplant, at flowering, and at fruiting. Each plant was scored individually for symptom severity on a 0-4 scale commonly used in the TYLCD community (Lapidot and Friedmann, 2002), and observations were averaged to obtain a single severity score for each variety.

Pictoral symptom severity cards, shown in Illustration 3.1 and Illustration 3.2, were given to each trial manager to facilitate the accurate identification of TYLCD symptoms. The symptom severity scale differentiates classes of symptoms as follows:

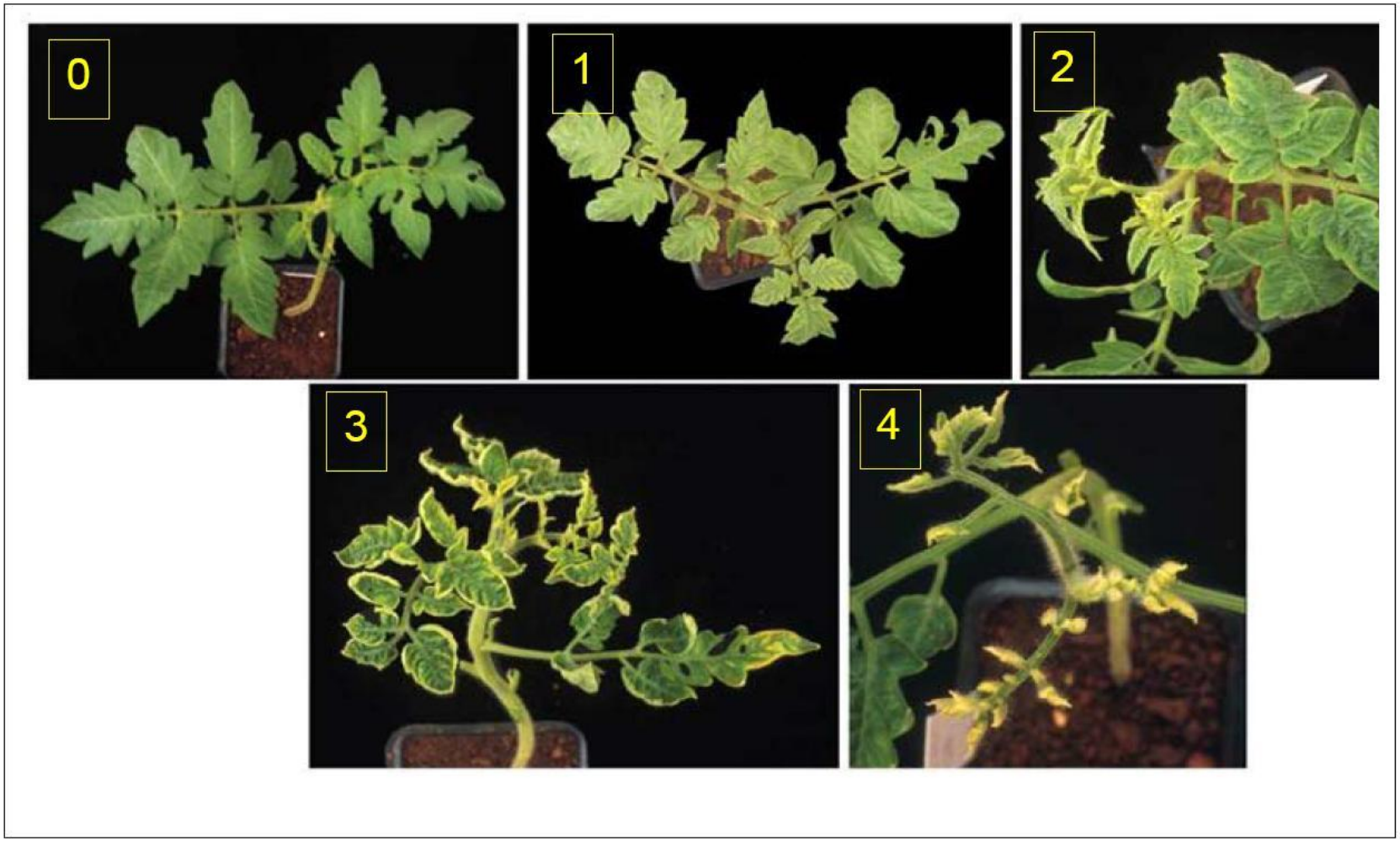

Illustration 3.1 – TYLCD symptom severity scale (Lapidot and Friedmann 2002)

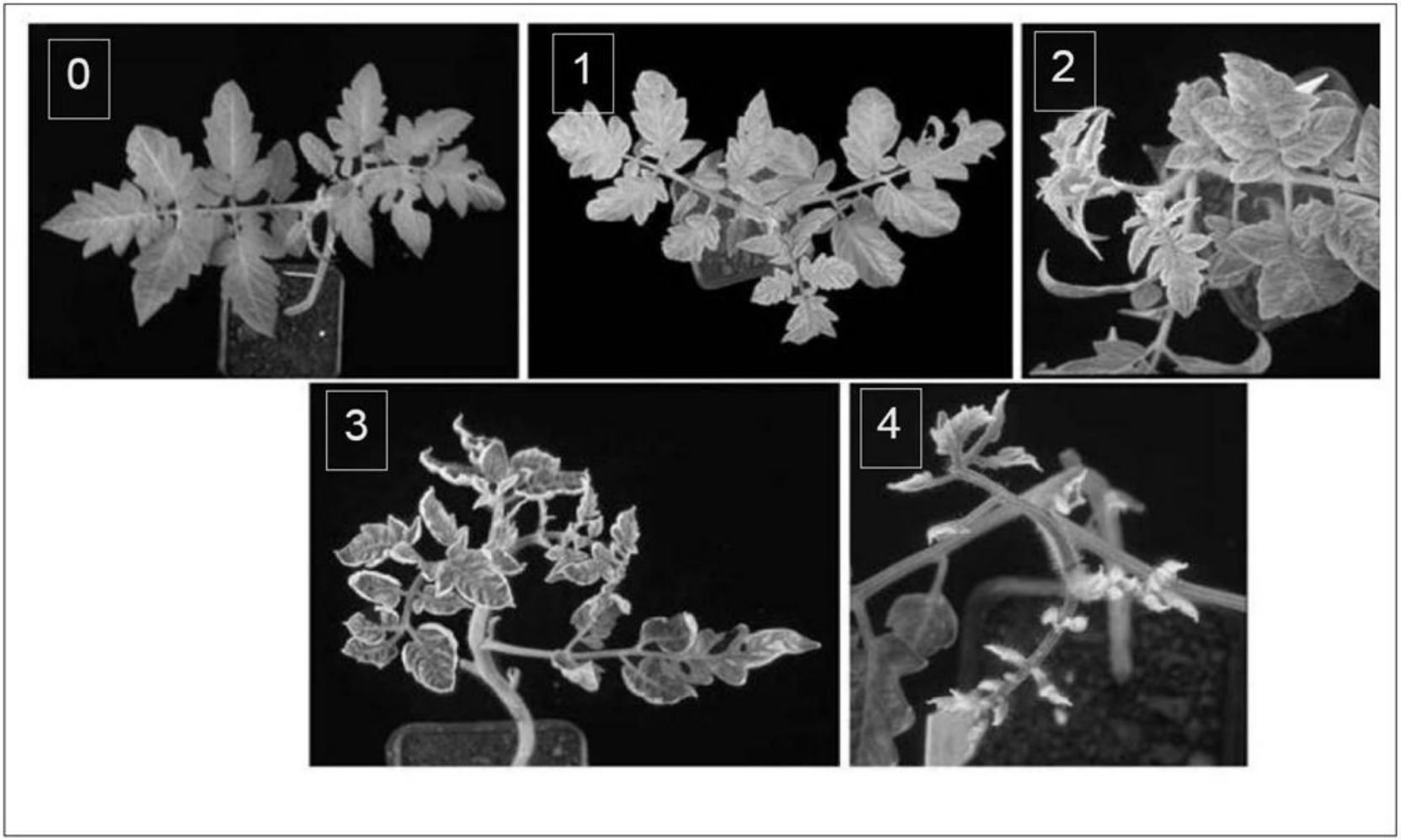

Illustration 3.2 – TYLCD symptom severity scale (Lapidot and Friedmann 2002)

0 No symptoms

1 Very slight yellowing of leaf edges

2 Yellowing and curling of leaves

3 Marked yellowing, curling, and cupping of leaves, accompanied by a continuation of plant growth

4 Severe stunting, curling, and cupping of leaves, accompanied by a cessation of plant growth

Efforts were made to train trial managers in the differentiation of TYLCD symptoms from heat and water stress, which can cause similar symptoms in plants. This is particularly problematic in West Africa, where one of the regional TYLCD-associated species (ToLCMLV) causes purple veining, a typical symptom of water stress.

Some research partners chose to calculate yields for each variety in the trial. Yields we initially calculated as kg per plot. To convert yields to kg/hectare, yield values were divided by the plot size (9.75 m^2), and then multiplied by 10,000 m^2/ha.

Characterization of Local TYLCD-Associated Viruses

An important component of the ABSPII project in West Africa was the characterization of the strains of tomato-infecting begomoviruses found throughout the region. This work was conducted by the lab of Robert Gilbertson at U.C. Davis, in cooperation with all of the West African APSPII research partners. During the tomato growing season, the research partners collected leaf samples from plants displaying TYLCD-like symptoms and squashed leaf discs onto nylon membranes. Squash blot hybridization was performed on the membranes at U.C. Davis to detect for the

presence of tomato-infecting begomoviruses. When relevant, the results of that work will be mentioned here to illustrate both the disease pressure present at a given trial, and the ability of the research partners to distinguish between symptoms of TYLCD and symptoms of other diseases or physiological stress responses.

Results

Benin (INRAB)

The trial in Benin was conducted at the INRAB Agonkamey research station near Cotonou, on Benin's Atlantic coast in the southern part of the country. Seeds were sown Nov. 2, 2005, and seedlings were transplanted to trial plots on Nov. 25, 2005. The trial ended Feb 6, 2006.

Several problems were encountered during the trial that limit the validity of the TYLCD severity scores reported by the partners in Benin. As a result of climate differences, the major season for tomato production in southern Benin is somewhat earlier than that of the more Sahelian regions of West Africa, where the tomato growing season typically runs from October through February. The rainy season in southern Benin begins as early as April and ends by July, making July through November the ideal tomato season in that region. By planting in November, at the end of the tomato season, the research partners in Benin inadvertently exposed their trials to elevated levels of pests and diseases that had accumulated in the region during the tomato growing season. As a result, the trial cultivars showed significant damage from root knot nematodes, Fusarium and bacterial wilts, spider mites, and *Helicoverpa armigera*. Since the trial managers were unaccustomed to the symptoms of TYLCD they appear to have scored the symptoms of many of these diseases and pests as

TYLCD. While the data from the trial (below) support this conclusion, further evidence can be gleaned from the squash blot samples prepared by the research partners in Benin for analysis at U.C. Davis. While all samples showing stunting and leaf yellowing were positive for begomovirus infection, the majority of the samples collected only showed leaf rolling, and tested negative for begomoviruses.

Figure 3.2 shows the development of TYLCD symptoms on the trial in Benin over the course of the growing season. Several things are evident from this graph. Firstly, symptom severity remained relatively weak throughout the course of the trial, with only a single variety (Chenoa) scoring a 3 by the fruiting time point, and the average variety scoring only a 2.25. Secondly, at any given time point the range of disease scores was extremely narrow, with the difference between the highest- and lowest-scoring varieties at fruiting being just 1 point on the symptom severity scale. Finally,

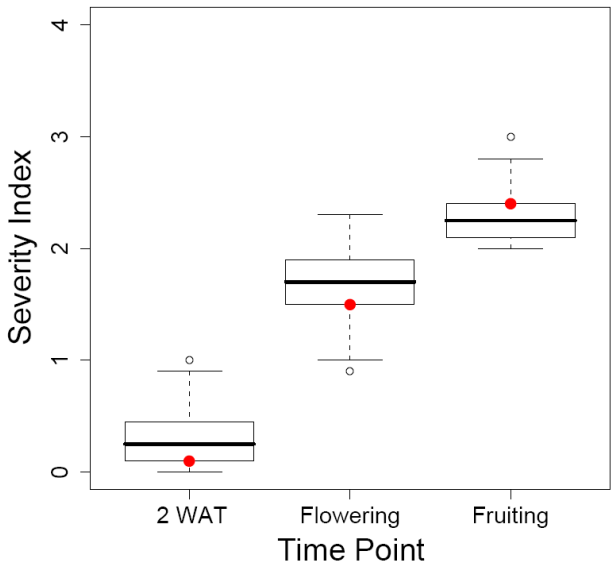

Figure 3.2 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points at the Agonkamey research station near Cotonou, Benin. Red dots represent the TYLCD symptom severity scores of Roma VF.

the susceptible check variety Roma VF did not perform as expected, falling in the lowest quartile of symptom severity observations at 2 weeks after transplant (WAT) and flowering, and failing to even be in the top ten varieties at fruiting. Based on these observations, it appears that one or several diseases were major constraints on tomato production in southern Benin during the trials, but there is no way to discern the effects of tomato-infecting begomoviruses from among all other diseases that were also interpreted to be TYLCD.

Given the strong disease and pest pressure seen on the trial in Benin, yields were very poor, and were therefore not reported.

Burkina Faso (INERA)

The trial in Burkina Faso was conducted in a major tomato growing area in the Kou Valley, near Bobo Dioulasso. Seeds were sown Dec. 3, 2005, and seedlings were transplanted Jan. 5, 2006. The trial ended in early April 2006.

Whitefly populations were observed to be very high in the trial region, with significant infestations occurring both on the agricultural research station and in farmers' fields. This may be due to the fact that the region is a significant producer of cotton, which is a preferred host of whiteflies. Squash blots of plants from the region with TYLCD symptoms were positive for begomovirus infection. TYLCD severity was very strong in the trial, with Roma VF developing a score of 3.6 by flowering and 4 by fruiting (Figure 3.3). A number of varieties demonstrated resistance to the virus, both through delayed development of symptoms and through an overall decrease in symptoms. Several varieties ended the trial with scores of less than 2.0 at fruiting, implying significant resistance: Thoriya, Atak, TY 75, Cheyenne E448, Chenoa, Bybal,

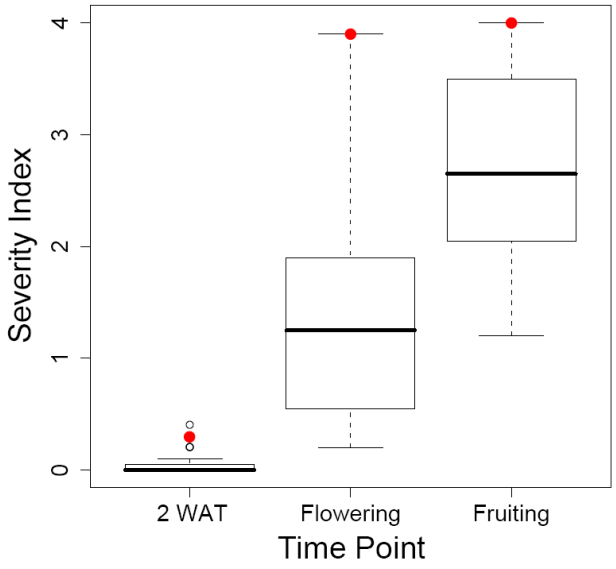

Figure 3.3 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in the Kou Valley, Burkina Faso. Red dots represent the TYLCD symptom severity scores of Roma VF.

Yassamen TH 99802, and Ponchita. An additional 15 varieties scored less than 3.0 at fruiting. Also interesting were varieties that showed delayed symptom development under TYLCV pressure, which often allows plants to develop higher yields before succumbing to virus symptoms. One particularly notable variety is Industry DR 10403, which, while scoring 2.9 at fruiting, developed the majority of its symptoms after flowering, having only scored a 0.3 at flowering. Due to this late onset of disease symptoms, Industry DR 10403 had one of the higher yields in the trial (7.6 t/ha) despite showing what would appear to be a significant susceptibility at fruiting.

Yields were reported for this trial, and ranged from 0.2 t/ha for Roma VF to 10.2 t/ha for Yassamen TH 99802, with a mean value of 4.4 t/ha. Yields were strongly negatively correlated with symptom severity (Figure 3.4). While the Burkinabe research partners did report a decrease in yield due to *Helicoverpa* and blossom end

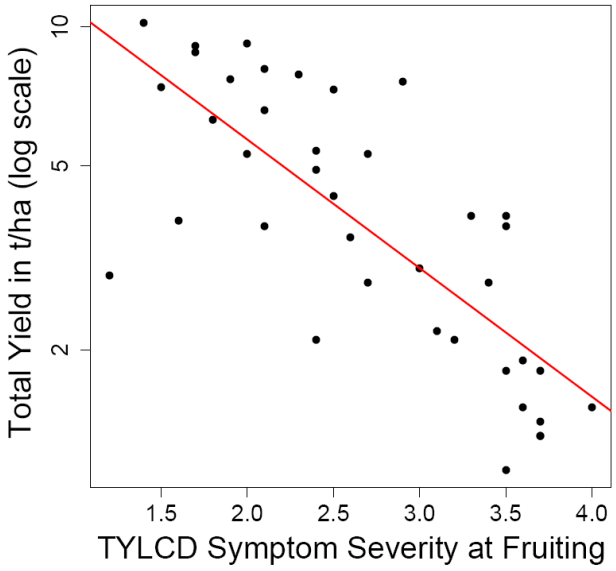

Figure 3.4 – Total yield plotted against TYLCD symptom severity at fruiting in Bobo Dioulasso, Burkina Faso. Slope = -2.50, R^2 = .59, p = 1.16e-08

rot, they pointed out that these very low yields were actually quite good for the region. TYLCD has become such a constraint in Burkina Faso that farmers have moved steadily southwards in an attempt to escape the high disease pressure accumulated in their original growing region. They argued what while 10 t/ha may seem low, to a tomato farmer in Burkina Faso it would make a big difference in livelihood.

Ghana (CRI)

The trial in Ghana was conducted twice consecutively in Kumasi, the second-largest city in the country. Kumasi is located towards the southern end of central Ghana, and thus has seasons similar to those of the Sahelian Zone to the north, but with generally higher humidity. Seeds for the first trial were sown Dec. 1, 2005, and seedlings were transplanted to the field on Dec. 21, 2005. During the first trial, several heavy rains created ideal conditions for the development of fungal diseases. Severe bouts of early blight and Fusarium wilt completely overpowered the TYLCD symptoms, making

scoring very difficult. Disease scores were collected at fruiting and flowering, and in addition yield data were collected, but prior to the fruiting stage the research partners decided to redo the trial following an application of fungicides. Therefore seeds for the second trial were sown on Feb. 4, 2006, and following an application of fungicides were transplanted to test plots on Feb 27, 2006. The trial ran through May 2006, and included 31 of the varieties that had been in the first trial. There were not enough seeds of the remaining 9 varieties: Atak, Chenoa, CLN 2123A, CLN2 2545B, F1 3019 Galina, Industry DR 10403, Lety F1, Ponchita, and PS 43316.

The second trial developed TYLCD pressure slowly, but by the fruiting stage the disease pressure was very strong, with Roma VF showing a symptom severity of 3.6 (Figure 3.5). Only three varieties emerged from the trial with disease scores below 2: FTC 6236, FTC 7351, and Yosra. Yields were not calculated as insufficient yields

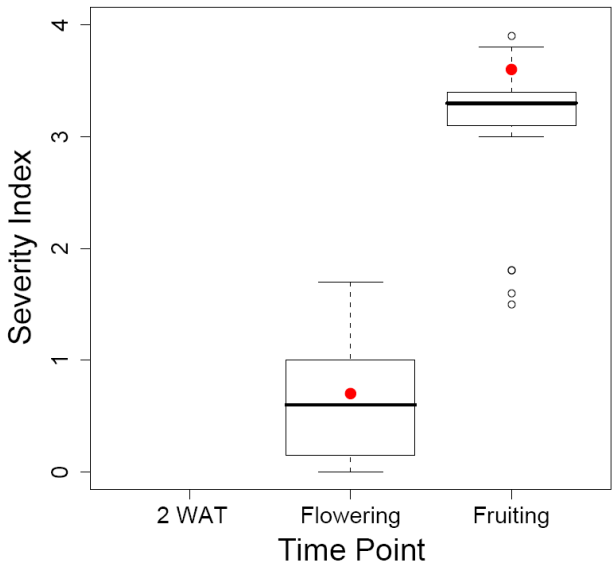

Figure 3.5 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Kumasi, Ghana. Note that no measures were taken at 2 WAT. Red dots represent the TYLCD symptom severity scores of Roma VF.

were produced. A number of non-fungal diseases and pests were observed on this trial, possibly confounding TYLCD severity measurement. They include root knot nematodes, bacterial wilt, and *Helicoverpa*.

Mali (IER) – Baguineda

One of the four trials in Mali was conducted at the IER Baguineda research station, which is located 20 km from Bamako, the capital of Mali, in a zone with a very typical Sahelian climate. Seeds were sown Jan. 16, 2006, and seedlings were transplanted just six days later on Jan. 22. (The research partners from IER in particular were found to have little experience with establishing seedling nurseries, an issue which was addressed in subsequent years.) The trial ended in April, 2006.

Squash blots of plant samples taken from the region confirm the presence of tomato-infecting begomoviruses, but disease pressure was relatively low in Baguineda (Figure 3.6). This was likely due to the host-free period conducted in July and August of that year, leading to slow accumulation of virus pressure. Analysis of the data shows that no significant symptom levels were observed in the trial until fruiting. At that time point, Roma VF did show one of the highest disease scores in the trial, but scored only a 2.7, indicating strong but not severe symptoms. Four varieties were scored as symptomless throughout the entire trial (Atak, Bybal, Chenoa, and Realeza) and 12 more varieties scored less than 1. Yield data were calculated and were quite low, ranging just from 1.1 to 6 t/ha, with a median value of 3.3. Yields were not statistically significantly correlated with TYLCD symptom severity.

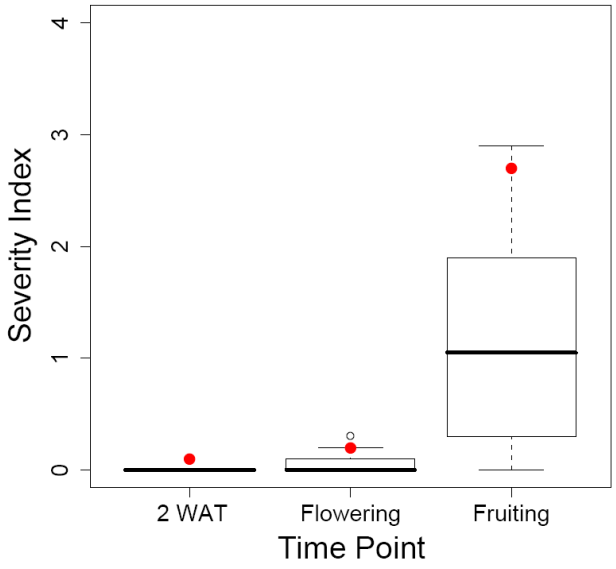

Figure 3.6 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Baguineda, Mali. Red dots represent the TYLCD symptom severity scores of Roma VF.

Mali (IER) – Cinzana, Sikasso and Sotuba

Research partners from IER established three further trials in areas around Mali. One was located in Cinzana, a region 35 km east of the city of Segou in eastern central Mali. A second was located in Sikasso, in southeastern Mali near the border with Burkina Faso and approximately 150 km from Bobo Dioulasso. Finally the last was located in Sotuba, a town on the outskirts of Bamako. All three trials experienced low disease pressure, and data were not shared.

Mali (AVRDC) – Samanko

The AVRDC regional headquarters in West Africa are based in Samanko, Mali, approximately 35 km from Bamako. The seeds for the trial at Samanko were sown Nov. 25, 2005, and seedlings were transplanted four weeks later on Dec. 20. Plots were fertilized prior to transplanting, and were managed as per AVRDC

recommendations. Plots were sprayed with deltamethrin every ten days at the rate indicated by the manufacturer for the control of *Helicoverpa*. TYLCD severity scorings were conducted on Jan. 6, 2006 (2 WAT), Jan. 30 (first flowering), and March 3 (first fruiting).

Whitefly populations were observed to be very high during the trial, and squash blots showed tomato-infecting begomoviruses to be present. Based on the symptom severity scores, it appears that TYLCD pressure increased gradually over the course of the season, and that cultivars in the trials showed a range of reactions from strong susceptibility to nearly complete immunity (Figure 3.7). At all three time points, Roma VF was one of the highest-scoring varieties, supporting the notion that TYLCD was the primary disease affecting the trials. At fruiting, one variety, Thoriya, showed no TYLCD symptoms, and nine additional varieties received scores lower than 1.0,

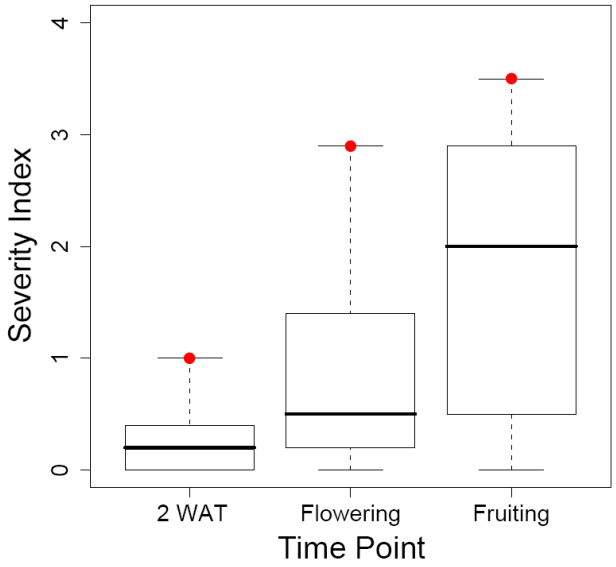

Figure 3.7 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Samanko, Mali. Red dots represent the TYLCD symptom severity scores of Roma VF.

indicative of very mild symptomatic responses: Bybal, Yassamen TH 99802, Ponchita, Lety F1, Chenoa, Atak, Yosra, Realeza, and FTC 6236. An additional eight varieties scored less than 2.0. Several varieties are also notable for slow development of TYLCD symptoms, despite eventually developing strong reactions to the disease. HA 3060 received a score of 2.8 at fruiting, but only 0.5 at flowering, and TLCV 15 had a score of only 0.2 at flowering, eventually developing to 3.2 at fruiting.

Yields were reported for the trial at Samanko, and were quite high compared with those from most other trials in the region. Yields ranged from 4.8 t/ha (CLN 1466J) to 23.4 t/ha (HMX 4810), with a median value of 15.6. A yield measure was not provided for Roma VF. There was a statistically significant negative correlation between symptom severity and yield; interestingly, the correlation was stronger for symptom severity at flowering than at fruiting, supporting the notion that symptoms developed early may have a stronger impact on yield than those developed after flowering.

Niger (INRAN)

The trial in Niger was conducted at the INRAN Gabagoura research station, approximately 15 km west of Niamey. The trial was planted in mid-December, but a low whitefly population led to an absence of TYLCD pressure on the trial. Squash blots collected from fields around the trial did show low levels of begomovirus infection, but showed no significant infestations. Other pathogens were observed, though, including *Helicoverpa*, root knot nematodes, Fusarium wilt, and bacterial wilt.

Senegal (CDH/ISRA)

The trial in Senegal was conducted at the Sangalkam research station in Rufisque, about 40 km east of Dakar on Senegal's Atlantic coast. Seeds were sown in Dec. 2005, and the trial concluded in March 2006. The locally popular variety Xina was included in the trial as an additional susceptible check.

A high incidence of TYLCD was observed in the fields surrounding the research station very early on in the trial. By local standards for tomato production the trial was planted late, and thus significant virus pressure had already built up in the surrounding area. Nonetheless, TYLCD incidence in the trial was only scored as moderate, with Roma VF and Xina ending the trials with scores of 2.9 and 3.2, respectively (Figure 3.8). Interestingly, the majority of symptom development occurred between 2 WAT and flowering, with only slight increases (and even occasional decreases) in severity

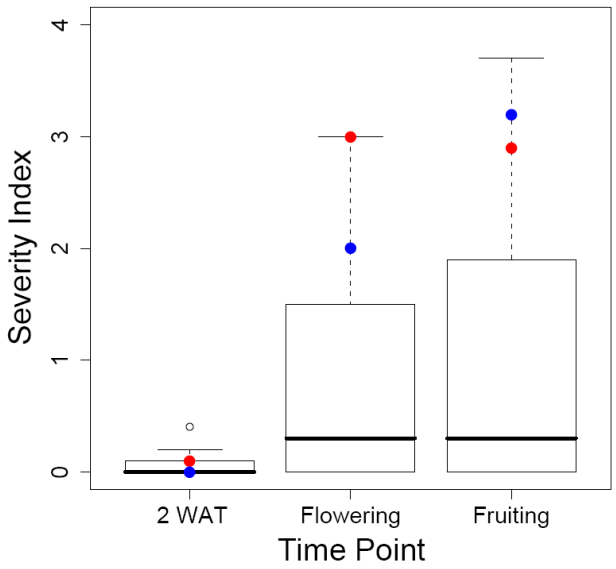

Figure 3.8 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Rufisque, Senegal. Colored dots represent the TYLCD symptom severity scores of susceptible checks: red = Roma VF, blue = Xina.

occurring between flowering and fruiting. This may reflect a decrease in viral inoculum in the surrounding region as the local tomato season ended and whitefly populations declined. Twelve varieties ended the trial with symptomless reactions: Atak, Bybal, Chenoa, Favi 9, FTC 6231, FTC 6236, Ponchita, Realeza, Thoriya, TY 75, Yassamen TH 99802, and Yosra. An additional 14 varieties scored less than 1.0. Yields were reported for the trial in Senegal and were quite high, with a few varieties surpassing even 60 t/ha. A significant negative correlation was observed between yield and TYLCD symptoms, with symptom severity scores at flowering and fruiting having equally significant correlations with yield.

Togo (ITRA)

In Togo, the research partners from ITRA conducted the trial at the Agbodrafo research station, approximately 30 km from Lomé, the nation's capital. The research station is in southern Togo, a coastal region known for extremely sandy soils. Seeds for the trial were sown on Nov. 10, 2005, and seedlings were transplanted Dec. 1.

TYLCD symptoms were observed on plants in the vicinity of the trial, and squash blots were positive for begomovirus infection. However, numerous other diseases and pests were also observed, including Fusarium wilt, bacterial wilt, spider mites, *Helicoverpa*, and root knot nematodes, which are particularly problematic in sandy soils. This may be a result of similar conditions to those observed in Cotonou, Benin, which is in the same climatic zone and only approximately 130 km away. While there is no immediate evidence that symptoms associated with other diseases were attributed to TYLCD in the Togalese trial, the data cannot be considered reflective of TYLCD incidence since the susceptible check received a lower severity score at fruiting than nearly half the varieties in the trial (Figure 3.9). However, several varieties did receive

particularly low symptom severity scores, implying decent performance in the face of whatever disease pressure may have been present. Varieties scoring less than 1.0 include Thoriya, Industry DR 10403, and Lety F1, and varieties scoring less than 2.0 include Sasya 0202 F1, Realeza, Nadira, Cheyenne E448, O4 108, Atak, CLN 2460E and CLN 2123A.

Very low yields were observed in Togo due to the wide range of disease and pest problems encountered, and yields were thus not reported.

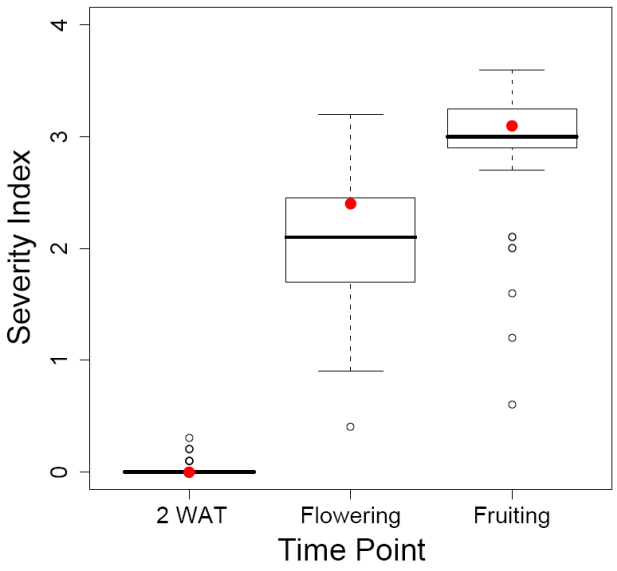

Figure 3.9 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Lome, Togo. Red dots represent the TYLCD symptom severity scores of Roma VF.

Discussion

Trial Location

A variety of difficulties were encountered during the preliminary TYLCD-resistant tomato trials conducted throughout West Africa in 2005-2006. Most notable among these were the prevalence of several diseases and pests, such as Fusarium wilt, bacterial wilt, root-knot nematodes, and spider mites, which could easily be confused with TYLCD symptoms and thus made TYLCD symptom severity scores from several locations difficult, if not impossible, to interpret. Conversely, in other locations, disease pressure from TYLCD was found to be too low to cause observable symptoms, diminishing the relevance of the trials.

Figure 3.10 shows the distribution of TYLCD symptom severity scores at fruiting for all trial locations. Two different types of distributions can be observed: Benin, Ghana, and Togo show very low variance, with the vast majority of observations falling within 1 symptom severity point, while Burkina Faso, both locations in Mali, and Senegal show much wider distributions, spanning 3 or more symptom severity points. In fact, these two groups correspond to the geographic distribution of the trials: trials in Burkina Faso, Mali and Senegal were all firmly within the semi-arid Sudano-Sahelian climatic zone, which was the primary target of this project, while the trials in Benin, Ghana and Togo were situated in the much wetter Guinean Zone (See Figure 3.1). The Guinean Zone has a different growing season than the Sudano-Sahelian Zone, with different climatic conditions and different pests and diseases that make the qualities of tomato cultivars adapted to the Sudano-Sahel very different from those adapted to the Guinean Zone. Trials conducted within the Guinean Zone were planted after the local tomato season had ended, exposing the cultivars under evaluation to a

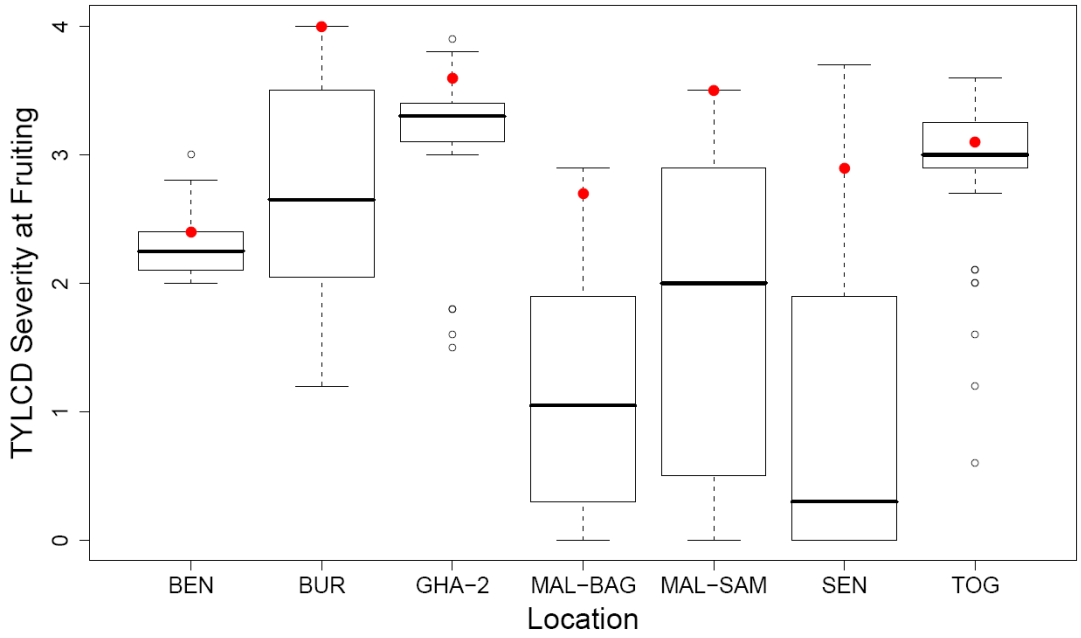

Figure 3.10 – Box and whisker plot of TYLCD symptom severity distributions for each trial location. Red dots represent variety Roma VF. Location codes: BEN: Benin; BUR: Burkina Faso; GHA-2: Ghana second trial; MAL-BAG: Baguineda, Mali; MAL-SAM: Samanko, Mali; SEN: Senegal; TOG: Togo.

range of pests, diseases, and weather conditions atypical for the tomato growing season. As a result, TYLCD symptom severity data from those trials could not be considered an accurate reflection of susceptibility to the disease, and were considered only secondarily when selecting the best-adapted varieties in the trial. The four trials with significant TYLCD pressure in the Sudano-Sahelian Zone – Burkina Faso, Mali-Baguineda, Mali-Samanko, and Senegal – will be referred to as "significant" trials for the remainder of this discussion.

Cultivar Selection

Table 3.2 shows a summary of the responses of all cultivars in all locations to TYLCD. It is clear that there was a range of responses, from strong TYLCD-resistance

Table 3.2 – Performance of putatively TYLCD-resistant tomato cultivars in the 2005-2006 preliminary screening trials. Numbers represent average TYLCD symptom severity scores at fruiting. For each trial, colors are allocated on a linear scale with yellow representing the lowest symptom severity score and blue representing the highest. Group A refers to the top 11 cultivar selections by the trial participants, and Group B refers to the next 10 selections. (cont'd on next page)

VARIETY NAME	BUR	MAL-BAG	MAL-SAM	SEN	Group	BEN	GHA-2	TOG
Atak	1.8	0	0.3	0	A	2.2		2.7
Bybal	1.5	0	0.1	0	A	2.3	3.8	3.2
Chenoa	1.6	0	0.3	0	A	3		3
Cheyenne E448	1.7	0.8	1.7	0.8	B	2.2	3.4	2.1
CLN 2123A	4	1.9	2.3	1.9		2.3		2.9
CLN 2460E	3.7	1.6	3.4	3.3		2.1	3.2	2.9
CLN 2468A	3.5		2.9	3.7		2.3	3.3	3.4
CLN 2498E	3.7		2.4	2.7		2.2	3.3	3
CLN 2545A	3.6	1.6		1.9		2.4	3.6	3.3
CLN 2545B	3.4		3	2		2.3		3.6
F1 3019 Galina	2.1	0.6	1.9	0.4	B	2.1		3.3
Favi 9	3.5	2.3	2.7	0		2.4	3.3	3
FTC 6231				0		2.4	3.3	
FTC 6236	2.3	1.9	0.5	0	B	2.2	1.6	3.3
FTC 7088	2.4		1.8	0.2		2.8	3	3.2
FTC 7127	3.5	1.9	3	2.5		2	3.1	3.2
FTC 7351	3.6	2.8	3.3	2.3		2.6	1.8	3.1
FTC 7483	2.7	0.4		1.6		2.3	3.3	3.2
GemPride	2.1	1.4	1.6	0.1	A	2.1	1.5	3.4
HA 3060	2	0.7	2.8	0.1	B	2	3.6	3

Table 3.2 (cont'd) – Location headings are: BUR – Burkina Faso; MAL-BAG – Baguineda, Mali; MAL-SAM – Samanko, Mali; SEN – Senegal; BEN – Benin; GHA-2 – Ghana, trial 2; and TOG – Togo.

VARIETY NAME	BUR	MAL-BAG	MAL-SAM	SEN	Group	BEN	GHA-2	TOG
HMX 4810	2.5		2	0.3	B	2.5	3.4	3
Industry DR 10403	2.9	0.7	1.3	0.3	A	2.4		1.2
Lety F1	3	0.2	0.3		A	2		0.6
Nadira	2.7	1.1	2.7	0.2	B	2.1	3.4	2.1
Nirouz TH 99806	2	0.8	1.6	0.2	B	2	3.4	3.4
O4 108	2.4	2	2.3	0.3		2.1	3.1	2.7
O4 240	3.2	0.9	3.1	0.5		2.1	3.3	3
O4 495	2.6	1	2	0.3	B	2	3.6	3
O4 498	3.1	1.2	1.8	0.8		2.2	3.1	3.2
O4 501	3.3	1.6	3.2	1.2		2.4	3.3	3.1
Ponchita	1.2	0.1	0.3	0	A	2.2		3
PS 43316	2.4	2.1	2.5	1.1		2		3.2
PT 4722A	3.7	2.9	3.3	1.8		2.4	3	3.3
Realeza	2.5	0	0.5	0	A	2.4	3.9	2
Roma VF	4	2.7	3.5	2.9		2.4	3.6	3.1
Sasya 0202 F1	3.5		2.8	0.6				2
Thoriya	1.9	0.2	0	0	A	2.1	3.3	1.6
TLCV 15	3.7	0.2	3.2	3		2.2	3.2	3
TY 75	1.7	0.3	1.1	0	B	2.5	3.5	3.4
Yassamen TH 99802	1.4	1.4	0.3	0	B	2.5	3.4	3
Yosra	2.1	1.5	0.5	0	A	2.3	1.8	3.6

to marked susceptibility, and that many varieties were relatively consistent in their responses across at least the four significant trials. While the table bases its comparisons on symptom severity scores at fruiting, several varieties are notable for other reasons. Industry DR 10403 and HA 3060, for instance, are two varieties that demonstrated resistance in the form of slow development of symptoms. Even when they did have high symptom severity scores at fruiting, both of these varieties were notable for low symptom severity at flowering. Yields are additionally relevant to this discussion. Yields were generally statistically significantly negatively correlated with symptom severity (Figure 3.11), confirming that TYLCD has an impact beyond just the development of symptoms. Interestingly, when compared across the four significant trials, yield was found to vary significantly by variety, though (perhaps not surprisingly, given the data) location was found to have a greater effect.

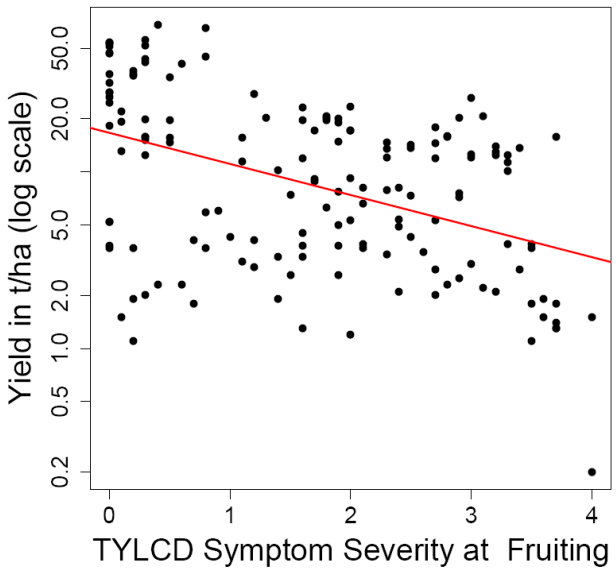

Figure 3.11 – TYLCD symptom severity at fruiting is significantly correlated with yield. Slope = -5.9, R^2 = .19, p-value = 1.44×10^{-8}

Cultivars were selected for inclusion in the following year's advanced trials at a meeting of the NARS trial managers in June 2006 in Bamako, Mali. Based primarily on demonstrated TYLCD resistance, but also based on yield, resistance to other diseases, or any other horticultural traits, each trial manager voted for their ten top variety choices from the trials. All varieties receiving more than four votes were selected for the following year's advanced trials (11 cultivars total). An additional 10 cultivars with two or three votes were selected as alternates, to be trialed in some (but not all) of the locations.

Recommendations for the 2006-2007 Growing Season

A number of changes were recommended based on the results of the preliminary trials of the 2005-2006 growing season. First and foremost, it was recommended that several trials be moved to locations more appropriate for a dry season tomato trial. Benin, Ghana and Togo all have very significant tomato-growing communities in their northern regions, which are situated within the Sudano-Sahelian Zone. These regions tend to be further from the headquarters of most agricultural research scientists, who are located in the southern regions near major administrative centers. Nonetheless, research partners were strongly encouraged to situate their trials in regions with tomato growing seasons that overlap with the trial dates. It was additionally recommended that the partners from Niger seek a more relevant trial site in a major tomato-growing region where disease pressure from TYLCD might be higher.

Recommendations were also made regarding the management of trials. Following the high incidence of pests and diseases in several trials in the 2005-2006 season, it was decided that more effort should be invested in protecting trials from these problems. Partners agreed to identify trial sites known to be free of soil-borne pathogens in the

following season, and committed to using chemicals when necessary to control pest infestations. Attention was also given to the establishment of seedling nurseries, and a protocol was developed to ensure more consistent practices between all participating countries.

Finally, numerous participants noted that 2 weeks after transplant was typically too early a time point for the observation of TYLCD symptom development. As a result, it was agreed that in the following year the three symptom severity time points would instead be flowering, fruiting, and first harvest.

The following chapter presents the results of the 2006-2007 advanced trial, which evaluated the materials selected in the 2005-2006 preliminary trial using a replicated design for greater statistical power.

CHAPTER 4

YEAR 2: ADVANCED TRIAL

Introduction

In 2006-2007 a series of advanced trials was conducted around West Africa to further evaluate the disease resistance and overall performance of tomato varieties selected during the preliminary screening trials of 2005-2006. A total of 23 varieties were included in the advanced trials, with the top 10 varieties from the 2005-2006 trials being evaluated in all participating locations, and with each of the remaining 13 varieties being trialed in whichever areas they had been most successful the previous year. As in the previous year, trials were conducted in seven countries, though for the advanced trials more careful attention was given to site selection within each country to ensure that trials were conducted in tomato-growing areas and during the tomato-growing season. The advanced trials incorporated a replicated design to allow for statistical analysis, and were scored for basic symptom severity, total yield, and marketable yield.

Materials and Methods

Plant Materials

23 tomato cultivars with proven TYLCD resistance were selected for inclusion in the 2006-2007 advanced trial (Table 4.1). These varieties were selected by all participating NARS partners based on performance in the previous years' trial. Seeds were again collected at AVRDC headquarters in Bamako, Mali, where they were repackaged and distributed to trial managers in each participating country. Contingent upon availability 200 seeds of each cultivar were sent each location.

Table 4.1 – Cultivars included in the 2006-2007 advanced trials and their sources. A check mark indicates inclusion in a particular trial. Country codes: BEN – Benin; BUR – Burkina Faso, GHA – Ghana; MAL – Mali; SEN – Senegal; TOG – Togo.

Seed Source	Variety Name	BEN	BUR	GHA	MAL	SEN	TOG
AVRDC	CLN 2764-99-13-18				✓		
	TLCV 15	✓					
De Ruiter Seeds	Bybal	✓	✓	✓	✓	✓	✓
	Industry DR 10403	✓	✓	✓	✓	✓	✓
	Lety F1	✓	✓	✓	✓	✓	✓
	Realeza	✓	✓	✓	✓	✓	✓
	Thoriya	✓	✓	✓	✓	✓	✓
Enza Zaden	Atak	✓	✓	✓	✓	✓	✓
	Chenoa	✓	✓	✓	✓	✓	✓
	Ponchita	✓	✓	✓	✓	✓	✓
	Yosra	✓	✓	✓	✓	✓	✓
Harris Moran	FTC 6236		✓	✓	✓	✓	
	FTC 7127	✓					
	FTC 7351			✓			
	HMX 4810		✓		✓		
Hazera	HA 3060		✓				✓
Seminis	Gempride	✓	✓	✓	✓	✓	✓
Syngenta	Cheyenne E448		✓				✓
	Nirouz TH 99806		✓		✓		✓
	Yassamen TH 99802		✓				✓
Takii	TY 75					✓	
Tropicasem	Nadira	✓					
	Roma VF	✓	✓	✓	✓	✓	✓

Trial Locations

Trials were conducted in eleven locations in seven countries throughout West Africa (Figure 4.1). Trials in Baguineda, Samanko, Sikasso and Sotuba, Mali and Bobo Dioulasso, Burkina Faso, were held on the same research stations as in the previous year. Trials in Benin, Ghana and Togo were moved from the southern regions to the

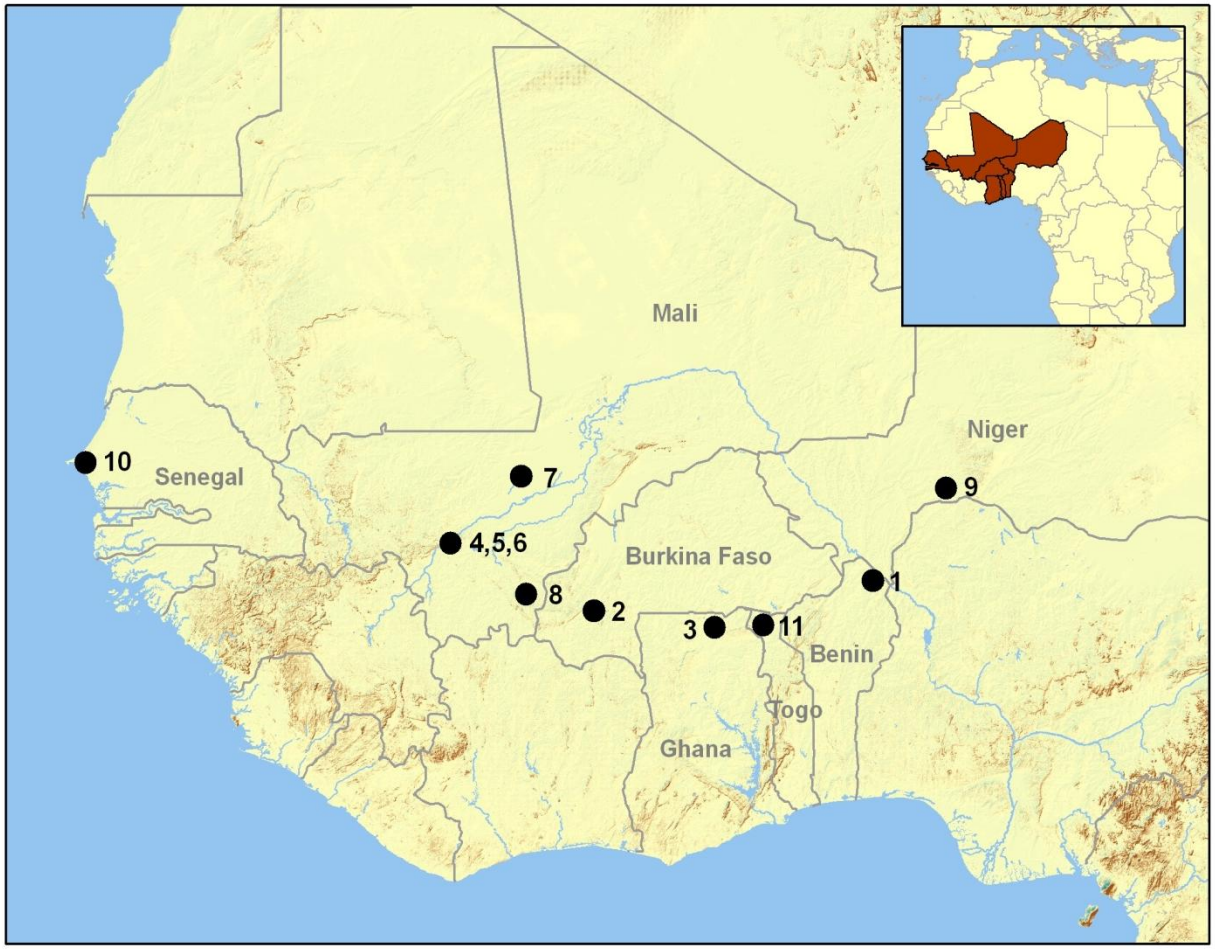

Figure 4.1 – Map of the 2006-2007 advanced trial locations. 1 – Kargui, Benin; 2 – Bobo Dioulasso, Burkina Faso; 3 – Navrongo, Ghana; 4, 5, and 6 – Baguineda, Samanko and Sotuba, Mali; 7 – Niono, Mali; 8 – Sikasso, Mali; 9 – Birni N'Konni, Niger; 10 – Rufisque, Senegal; 11 – Dapaong, Togo.

northern regions of those countries to ensure more appropriate climatic conditions. The trials in Cinzana, Mali and Niamey, Niger were moved closer to tomato-growing regions to increase the TYLCD pressure on the trials.

Starting in the project's second year, the author recorded GPS coordinates of all trial sites during visits. GPS coordinates of each trial location can be found in Appendix 3.

Trial Establishment and Management

In the previous year, many aspects of trial management were left up to the NARS research partners with the understanding that their management practices would likely mimic those of their local tomato farming communities and would thus make the trial results more locally relevant. Based on the results of the 2005-2006 preliminary screening trial, it was agreed that certain management practices should be adopted by all project participants to prevent the loss of trials to diseases other than TYLCD and to better evaluate the relatively subtle differences in yield potential and yield quality between the selected cultivars. Therefore the trial protocol for the 2006-2007 advanced trial included specific practices related to site selection, seedling nursery establishment, and field management. To minimize the impacts of some of the most devastating diseases observed in the previous year, the protocol specified that sites must be known to be free of soil-borne illnesses such as root-knot nematodes, *Fusarium oxysporum* f.sp. *lycoperisci*, and *Ralstonia solanacearum*. Seedling nurseries were to be established as per the protocols of the NARS, as in the previous year, but the trialing protocol specified that healthy seedlings would be transplanted three to four weeks after germination to prevent the trial managers from transplanting early and in the process unwittingly exposing fragile seedlings to the field environment before they reached maturity. Fertilization practices were also specified:

both mineral fertilizers (i.e. NPK) and organic matter (farmyard manure or compost) were to be incorporated into the soil in advance of transplanting, and top dressing with NPK was recommended during the growing season. Finally, the protocol specified that *Helicoverpa armigera* and other insect pests would be controlled by regular applications of selective pesticides, and that weeds would be controlled regularly.

Plot layout was similar to the previous year's, with 26 plants per elementary plot being laid out in two rows of 13 plants each. Spacing was again 0.6 m between rows, and 0.5 m within rows. However, unlike the previous year's preliminary trial protocol, the advanced trial protocol included three replications following a randomized complete block design in which all varieties were included in all blocks, and in which the placement of the elementary plots within each block was randomized. Due to space constraints, only one plot of Roma VF was included in each block.

Disease severity scoring and yield calculations

Disease scoring was done at three time points during the growing season and according to the same symptom severity scale used in the 2005-2006 trials (Illustration 3.2). However, due to uniformly low symptom severity scores at 2 weeks after transplanting in the first year's trial, the three time points were shifted later, to flowering, fruiting, and first harvest. In addition to scoring for symptom severity, trial managers were also to calculate both total and marketable yields (i.e. only undamaged fruit) in t/ha or kg/ha based on eight total harvests. Since in the follow-up to the previous year's trial many trial managers had also expressed an interest in seeing more yield-related measures such as fruit size and weight and fruits per plant or fruits per cluster, many trials reported such measures for the 2006-2007 advanced trial.

Yields were statistically analyzed by ANOVA for significant differences due to variety, block, and disease severity. All statistical analyses were performed in R. When necessary, yield data were transformed by a Box-Cox transformation to ensure normality of the data. Tukey HSD was used as a multiple-testing method for determining which yields were statistically significantly different from each other.

Results

Benin (INRAB)

The advanced trial in Benin was conducted in the village of Kargui, in the far north of the country near the city of Malanville and the borders with Burkina Faso and Niger. Seeds were sown on Nov. 20, 2006, and seedlings were transplanted on Dec. 18. First harvest took place on March 15, 2007, with the final harvest taking place April 20.

The Beninoise advanced trial was of significantly higher quality than the previous year's preliminary trial. This is due in part to its location – Kargui has a climate typical of the Sudano-Sahelian zone and is thus an ideal location for dry season tomato production, whereas the previous year's trial had been in the humid southern zone where various diseases severely limit production in October through March. However, the higher quality of the trial was also likely associated with improved management practices. The Beninoise research partners fertilized their plots before transplanting, and again around flowering. In addition they applied carbofuran to prevent nematode infestation, and fungicides and insecticides to address fungal and insect problems as they arose during the growing season. The result is that no diseases and pests other than TYLCD became significant problems during the Beninoise advanced trial.

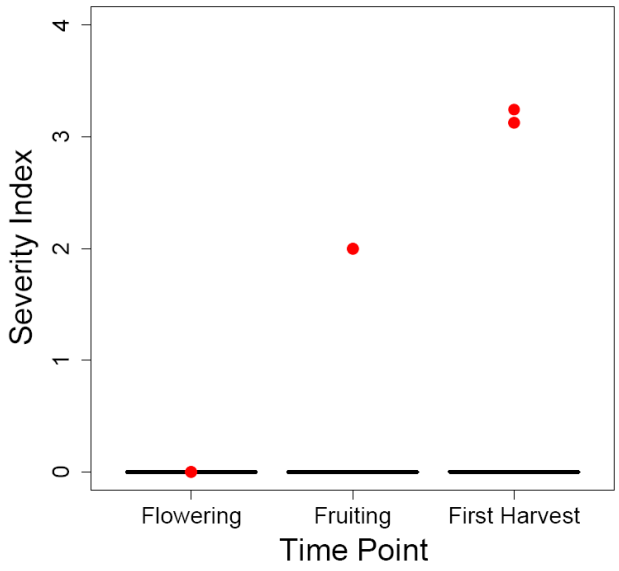

Figure 4.2 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Kargui, Benin. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Based on the distribution of TYLCD symptom severity scores (Figure 4.2) it is clear that the cultivar responses to TYLCD in Benin were bimodal, either expressing complete resistance or complete susceptibility. In fact, the only cultivar to develop symptoms during the trial was the susceptible check Roma VF, which at first harvest had an average symptom severity score across the three blocks of 3.2, implying moderately strong disease pressure. Observations of whitefly population levels and disease severity symptoms during the trial confirm the presence of TYLCD pressure that was sufficient for screening but not severe.

Yields for the trial were quite high, ranging from a maximum of 46.5 t/ha (Yosra) to a minimum of 19.1 t/ha (Roma VF)**.** Interestingly, marketable yields were exceedingly close to total yields, differing from them by at most 1.4 t/ha – this is likely a further indication of the excellent management practices of the trial managers. ANOVA

showed that yields varied significantly by variety, and a Tukey HSD test showed that three varieties (Yosra, Industry DR 10403, and Realeza) had significantly higher total yields than the lowest-scoring variety, the susceptible check Roma VF.

Burkina Faso (INERA)

The advanced trial in Burkina Faso was again conducted in the Kou Valley near Bobo Dioulasso, and again TYLCD pressure was extremely high. Seeds for the trial were sown on Nov. 22, 2006 and transplanted on Dec. 22. The trial partners decided to do four repetitions instead of three since they had enough space and enough seeds.

Whitefly populations were very high in the trial, and TYLCD symptoms were observed to be very high in neighboring fields early on in the trial. However, root-knot nematodes and bacterial wilt were also observed, and appeared to be overwhelming the TYLCD symptoms at earlier stages of the trial.

The TYLCD symptom severity distribution plot (Figure 4.3) shows a steady increase in symptom severity over the course of the season. At flowering essentially no symptoms were visible on any plants, but by fruiting there was a range of responses, from completely symptomless (one plot of Atak) to a score of 3.4 (one plot of Realeza – Roma VF had an average score of 3.0 across all four blocks). By first harvest there were no symptomless plants, and scores ranged from 2.1 (single plots of Nirouz TH 99806 and Bybal) to 4.0 (Roma VF in three plots). The symptom severity data at first harvest are extremely narrowly distributed and therefore difficult to interpret, but at fruiting three varieties had an average symptom severity score below 1: Atak, Bybal, and Yosra. Two additional varieties had symptom severity scores below 1.1: Yassamen TH 99802, and Ponchita.

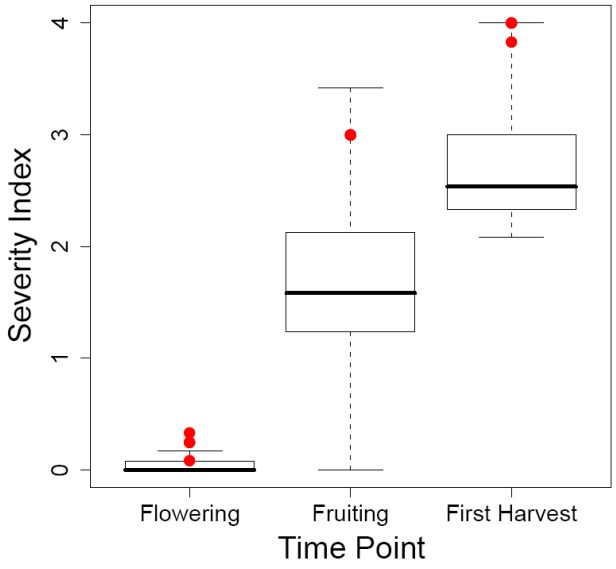

Figure 4.3 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in the Kou Valley, Burkina Faso. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Average yields in the trial were much better than those seen the previous year, ranging from 4.4 t/ha for Roma VF to 23.4 t/ha for Industry DR 10403. Analysis of variance shows that yield differences between varieties were significant, but also that yield comparisons between blocks were highly significant. Figure 4.4 shows that yields increased from block 1 to block 4 in a highly significant manner. Similarly, Figure 4.5 shows that TYLCD symptom severity measures varied between blocks, with block 1 being the highest and block 4 being the lowest. One possible explanation for this observation is that there was a TYLCV pressure gradient across the trial, with many more viruliferous whiteflies approaching the trial from one side than the other. This is in fact highly plausible – in a field adjacent to the trial site, a farmer had allowed old

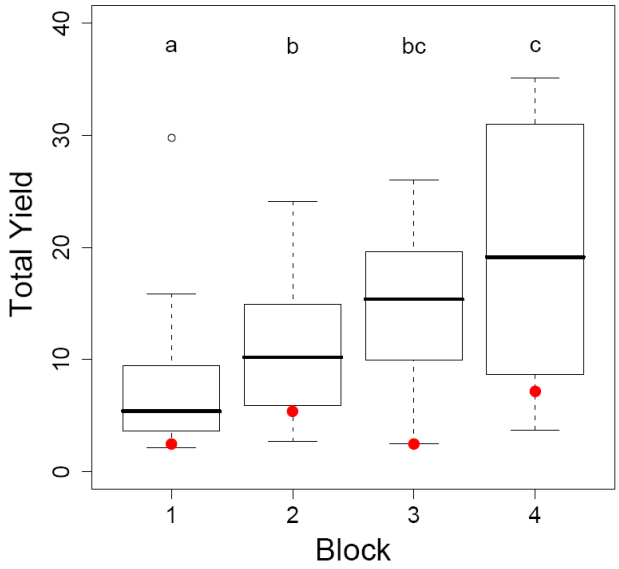

Figure 4.4 – Box and whisker plot of the distribution of total yield measurements across four blocks in the advanced trial in the Kou Valley, Burkina Faso. Letters above each box plot indicate statistically significant groupings at $p < .05$. Red dots represent the susceptible check cultivar Roma VF.

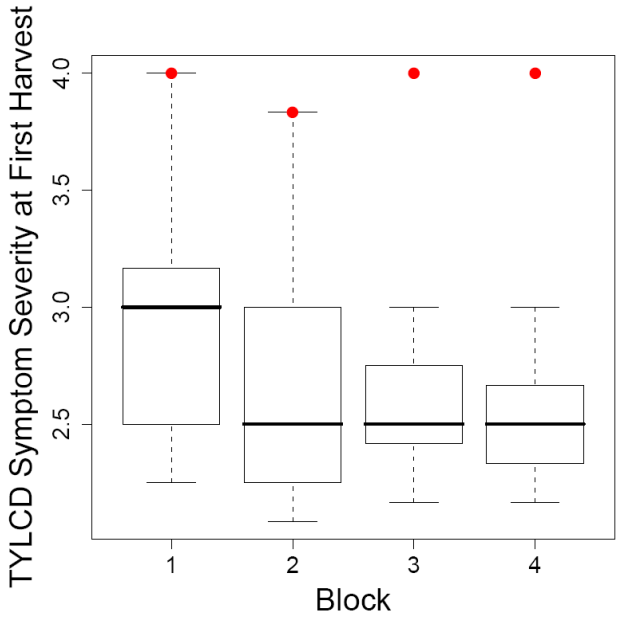

Figure 4.5 – Box and whisker plot of the distribution of TYLCD symptom severities at first harvest across the four blocks of the advanced trial in the Kou Valley, Burkina Faso. Red dots represent the susceptible check Roma VF.

tomato plants to continue growing, rather than plowing them under. As a result, those tomato plants were likely serving as a reservoir for TYLCV inoculum, placing higher disease pressure on the block closest to the infested field than on the block located farthest from it. An equally plausible explanation, however, is that the field had a gradient of soil-borne pathogens, and that symptoms from soil-borne illnesses were being confused with TYLCD symptoms. In either case, the advantage of the replicated trial design is very evident – without blocking, the gradient effect would not have been detectable and would have influenced comparisons of yields between cultivars.

A Tukey HSD test showed that, when accounting for the effects of both variety and block, two varieties had significantly higher yields than Roma VF, the lowest yielding variety. They were Industry DR 10403, and Nirouz TH 99806.

The research partners from Burkina Faso conducted a root knot nematode count on all varieties to see if they could discern any levels of resistance. Varieties were scored on scale of 1-5 based on the number of root nodules observed. Three varieties had scores lower than 2 – Atak, HA 3060, and Industry DR 10403 – and one variety, FTC 6236, had a score of less than 1, implying highly significant nematode resistance.

Ghana (CRI)

The advanced trial in Ghana was conducted in Navrongo, in the Sudano-Sahelian northern region of the country very near the border with Burkina Faso. Seeds for the trial were sown on Nov. 14, 2006, and seedlings were transplanted to the field on Dec. 11. The first harvest was conducted very late, on March 15, 2007, and the last harvest was conducted on March 30. The trial partners were in the midst of an internal review during the trial, and were not available to visit the trial plot regularly. Evidently the

technicians overseeing the trial significantly delayed harvest in the hopes that their superiors would come to see the trial prior to harvest. As a result, many fruits were damaged by rodents while still on the vine, and thus marketable yield was significantly lower than total yield. One interesting outcome, though, was the discovery that the fruits of FTC 6236 have very significant longevity on the vine, leading that variety to have the highest yields in the trial. (No significant differences were found between varieties for yield, however.)

TYLCD incidence in the Ghanaian advanced trial was quite high (Figure 4.6). While disease pressure at flowering was very mild, by fruiting some varieties were showing marked symptoms, with Roma VF averaging 2.1 on the symptom severity scale. Roma VF ended the trial at first harvest with a symptom severity score of 3.7, implying very high disease pressure. Nonetheless several varieties performed very well under the pressure, with Lety F1 and Bybal scoring below 1, and Thoriya, Industry DR 10403, Ponchita, FTC 6236, Gempride and Chenoa scoring below 2.

As noted previously, yield was not found to differ significantly between varieties. Yields were quite good across the whole trial, ranging from 40.2 t/ha for FTC 6236 to 18.8 t/ha for FTC 7351. Marketable yields ranged from 32.0 t/ha for FTC 6236 to 9.9 t/ha for FTC 7351, and were also found to not vary significantly between varieties.

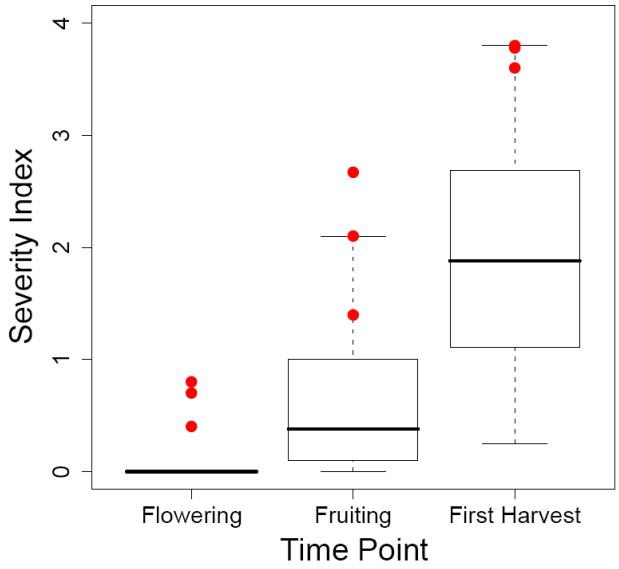

Figure 4.6 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Navrongo, Ghana. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Mali (IER) – Baguineda, Niono, Sikasso and Sotuba

The research partners at IER conducted advanced trials in four locations throughout Mali. Three of the four (Baguineda, Sikasso, and Sotuba) were also trial sites in the previous year's preliminary trials, while Niono was selected as a new site, approximately 85 km north of Segou. While the trials in Sikasso, Sotuba, and in particular Niono were well-managed, they developed no TYLCD symptoms and therefore no data from those trials were shared. In contrast, the trial in Baguineda did develop significant levels of the disease, with the susceptible check Roma VF showing a symptom severity score of 4.0 by first harvest (Figure 4.7). However, it is relevant to note that Baguineda had implemented a host-free period prior to the tomato growing season that year – it was likely only due to the relatively late planting (November vs. September) that significant TYLCD pressure developed in the trial. Disease pressure was low at flowering, but by fruiting the majority of the symptom development in the

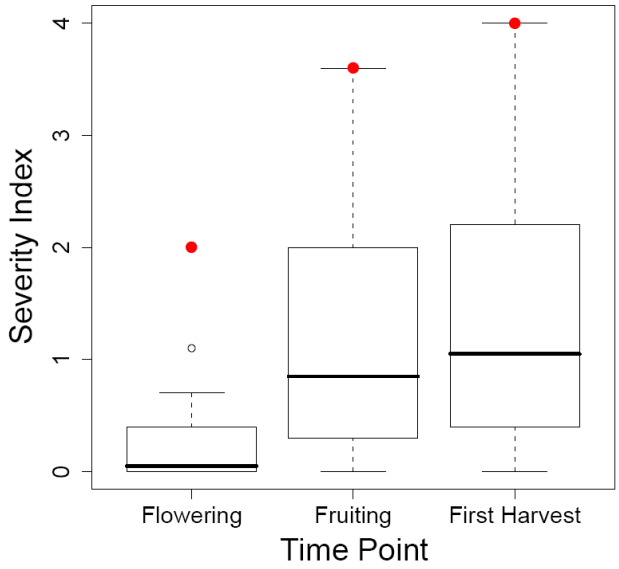

Figure 4.7 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Baguineda, Mali. Red dots represent the TYLCD symptom severity scores of Roma VF.

trial had already occurred, with minimal increases taking place between fruiting and first harvest. Varieties in the trial showed a range of reactions, from symptomless through significant susceptibility. Two varieties, Atak and Yosra, remained symptomless at first harvest, and four more showed symptom severity levels below 1.0 (Chenoa, Lety F1, Ponchita and Realeza). Yields were reported for the trial, and ranged from 25.5 t/ha for Lety F1 to 44.7 t/ha for Cheyenne E448.

Mali (AVRDC) – Samanko

AVRDC again conducted a trial at the Samanko research station. Disease pressure was moderately high, with Roma VF developing an average TYLCD symptom severity score of 3.3 at first harvest (Figure 4.8). All other varieties in the trial remained mostly symptomless, with the highest disease score (0.3) developing on CLN 2764-99-13-18,

an AVRDC breeding line trialed only in Samanko and not included in the previous year's preliminary trial.

Total yields for the trial were quite high. Interestingly, Roma VF showed the highest average total yield of 39.6 t/ha, while Lety F1, a cherry tomato, showed the lowest yield of 19.5 t/ha. This may be indicative of a combination of good management practices and late onset of TYLCD. Yields did not vary significantly between varieties, but blocks did have a significant impact on yields (Figure 4.9). This was likely due to an irrigation problem – drip irrigation was used, but a line was clogged, preventing blocks from being watered equally. This additionally led to the development of blossom end rot in some blocks, which along with high levels of sun scorch caused marketable yields to be very low for the trial.

Niger (INRAN)

The INRAN advanced trial in the 2006-2007 growing season was conducted on a research station in Birni-N'Konni, a town in southern Niger approximately 350 km east of Niamey and less than 10 km from the border with Nigeria. Birni-N'Konni is in a region best known for its onions – the most popular onion variety in all of West Africa, Violet de Galmi, is named for the town of Galmi just 35 km to the east. Tomatoes are also an important crop in the region, however, and since low TYLCD incidence was observed in Niamey the previous year the NARS partners from Niger decided to move their trial to Birni N'Konni. Seeds for the trial were sown on Nov. 16, 2006, and transplanted Dec. 23.

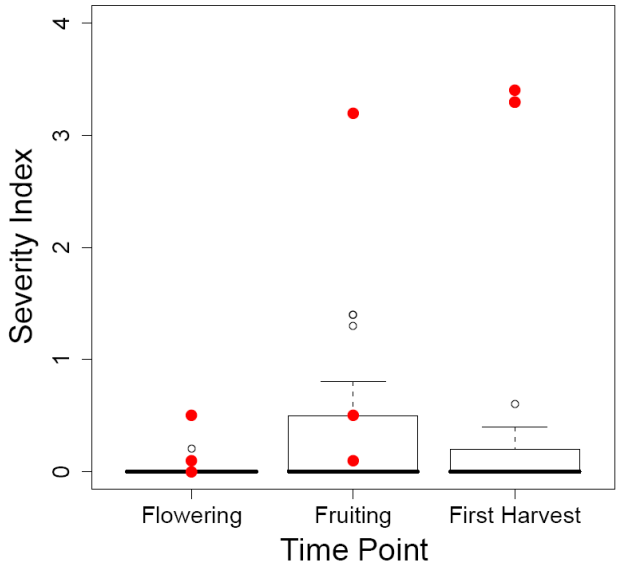

Figure 4.8 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Samanko, Mali. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

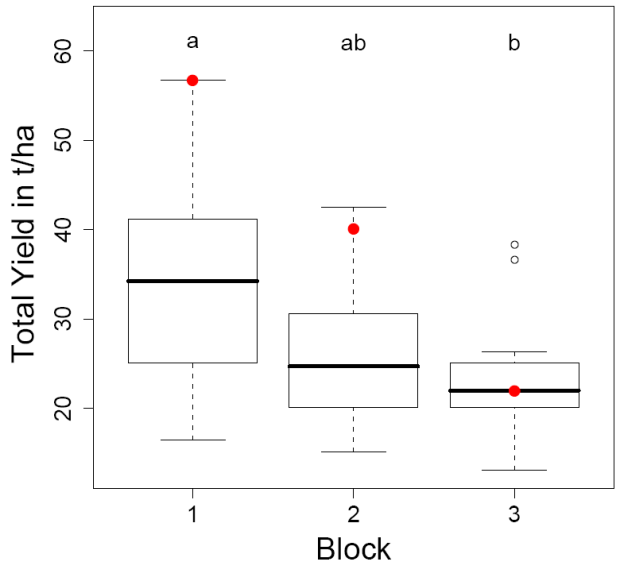

Figure 4.9 – Box and whisker plot of the distribution of total yield measurements across the three blocks in the advanced trial in the Samanko, Mali. Letters above each box plot indicate statistically significant groupings at $p < .05$. Red dots represent the susceptible check cultivar Roma VF.

While whitefly populations were observed to be moderately high at the Nigerien advanced trial, TYLCD pressure was very low and no significant symptom levels developed during the course of the trial. In the nearby valley of Dogueraoua, in which tomatoes are a major crop (though secondary to onions), TYLCD symptoms were observed but were not seen to be a major constraint, with *Helicoverpa* and root-knot nematodes causing much more damage.

Yields were reported for the trial, and ranged from 58.4 t/ha for Atak to 33.0 t/ha for Lety F1. Since Lety F1 is a cherry tomato, it is relevant to note that the cultivar with the next-lowest yield was Roma VF, with 39.1 t/ha. There was no statistically significant yield difference between cultivars in the trial.

Senegal (CDH/ISRA)

The Senegalese advanced trial was again conducted at the Sangalkam research station in Rufisque, about 40 km east of Dakar. Seeds were sown Nov. 14, 2006, and seedlings were transplanted on Dec. 19. The trial was well managed, with a balanced fertilizer applied at transplanting, and again in mid-January. A fungicide was applied for *Alternaria solani* in mid-January, and Iprodione was applied for nematodes at the end of February. First harvest was on Feb. 21, 2007, and harvest went through April 17. TYLCD observations were conducted on Jan. 31, Feb. 21, and March 12 – thus the second observation coincided with first harvest, and the third observation was actually halfway through the harvest period.

TYLCD pressure at the Senegalese advanced trial was moderate to strong, with both Roma VF and the local susceptible cultivar Xina gradually developing symptoms over the course of the growing season, eventually reaching 3.7 and 4.0, respectively, at

mid-harvest. However, no other varieties showed any disease symptoms at any time points (Figure 4.10).

Yield data were reported, and ranged from 55.9 t/ha for either Roma VF or TY 75 to 17.9 t/ha for Xina., with those two yields being significantly different from one another. Unfortunately, there was an irreconcilable discrepancy between the original data tables and a summary table of the data, making it difficult to know if Roma VF or TY 75 was the cultivar to yield 55.9 t/ha.

Togo (ITRA)

In 2006-2007 the Togalese trial was moved from the southern city of Lomé, where it had been the previous year, to the Tantiégou research station in the northern city of Dapaong, near the border with Burkina Faso. Dapaong falls within the Sudano-

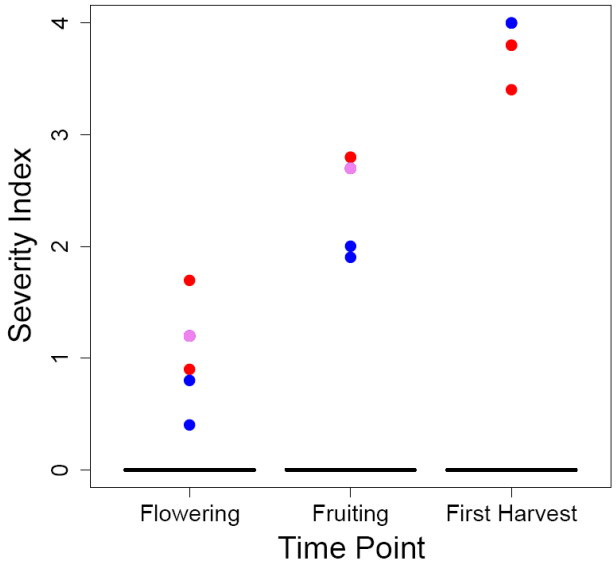

Figure 4.10 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Rufisque, Senegal. Colored dots represent the TYLCD symptom severity scores of individual plots of the susceptible checks: red = Roma VF, blue = Xina, purple = both Roma VF and Xina.

Sahelian climatic zone, and the trial was thus conducted during the local tomato-growing season.

Mild TYLCD pressure was observed in the Togalese trial, but it appears to have been overwhelmed by the symptoms of at least one other disease, possibly early blight. Additionally, in mid-February trial technicians were found to have been spraying pyrethroid insecticides to control insect populations during the trial, thereby inadvertently reducing whitefly populations and the spread of tomato-infecting begomoviruses. These technicians were instructed to stop spraying the insecticides, but it was probably too late: Roma VF had developed a symptom severity score of only 2.0 by the first harvest (Figure 4.11). Mild TYLCD symptoms did develop on several other cultivars, while still others remained mostly symptomless. Atak, Chenoa, Industry DR 10403, and Realeza all shared the lowest symptom severity score of .33 at first harvest, and Lety F1, Thoriya, and Nirouz TH 99806 all had scores below 1.0.

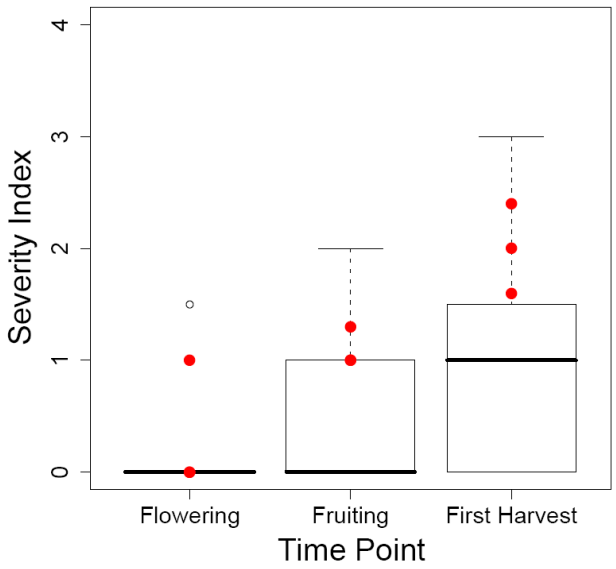

Figure 4.11 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Dapaong, Togo. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Yields were reported for the Togalese advanced trial, and ranged from 20.2 t/ha for Realeza to 46.0 t/ha for Industry DR 10403. Roma VF had the second-lowest yield of 20.4 t/ha. No statistically significant differences were found between the yields of any of the tested cultivars.

Discussion

TYLCD Resistance

The varieties included in the 2006-2007 advanced trials had previously undergone one round of selection for TYLCD resistance during the 2005-2006 preliminary screening trials. As a result, it is no surprise that all varieties in the trial showed significantly higher resistance to TYLCD than the susceptible check Roma VF, evidenced both by low symptom severity scores and high yields. Under the moderately strong disease pressure seen in some trials, including those in Benin, Samanko, Mali, Senegal, and Togo, no varieties under evaluation showed any susceptibility to TYLCD. Importantly, this does not imply that TYLCD pressure was low – in all of those trials, with the possible exception of that in Togo, the susceptible check Roma VF developed significant TYLCD symptoms during the course of the trial, showing that for locally popular tomato varieties, TYLCD is still a major constraint to production.

Among the collection of resistant cultivars, it is difficult to discern differences in the levels of resistance based on the data from the advanced trial. Table 4.2 shows the symptom severity scores at first harvest from Burkina Faso, Ghana, and Baguineda, Mali, all of which experienced high enough TYLCD pressure during the trial to induce symptoms on some of the test cultivars. Aside from the marked susceptibility of Roma

Table 4.2 – TYLCD symptom severity scores at first harvest in Burkina Faso (BUR), Ghana (GHA) and Baguineda, Mali (MAL-BAG). Cell colors range from yellow (lowest score in each location) to blue (highest score in each location).

VARIETY NAME	BUR	GHA	MAL-BAG
Atak	2.25	2.07	0.00
Bybal	2.39	0.96	1.00
Chenoa	2.48	1.93	0.40
Cheyenne E448	2.65		2.70
FTC 6236	2.52	1.47	
FTC 7351		3.23	
Gempride	2.81	1.52	1.20
HA 3060	2.65		2.20
HMX 4810	2.58		2.60
Industry DR 10403	2.70	1.21	2.10
Lety F1	2.80	0.81	0.50
Nirouz TH 99806	2.44		
Ponchita	2.53	1.27	0.60
Realeza	2.96	2.50	0.30
Roma VF	3.96	3.73	4.00
Thoriya	3.06	1.17	1.10
Yassamen TH 99802	2.48		
Yosra	2.40	3.53	0.00

VF, no other obvious patterns are evident. A few varieties, such as Ponchita and Chenoa, were neither the most resistant nor the least resistant cultivars in any of the three trials. The majority of the cultivars, however, performed exceedingly well in at least one of the three trials and exceedingly poorly in another. Yosra, for instance, had the second lowest TYLCD symptom severity score of all varieties in Baguineda, and the third lowest score in Burkina Faso, but had the highest score of any variety other than Roma VF in Ghana. Similarly, Realeza had the third lowest symptom severity score in Baguineda, but the second highest score in Burkina Faso and the third highest score in Ghana. There are several potential explanations for these discrepancies. It is plausible that different species of tomato-infecting begomoviruses are prevalent in each location, and that varieties in the trial carried varying levels of resistance to

different strains of tomato-infecting begomoviruses, leading to different responses by the same cultivars in different regions. Further surveys of local virus populations would be necessary to confirm this possibility. An alternative explanation is that symptom severity scoring continued to be confounded by other diseases and stresses during the advanced trials of 2006-2007, and that diseases not associated with begomoviruses were responsible for at least some of the symptoms described as TYLCD on cultivars under evaluation. In either case, it is evident that all varieties in the advanced trial were resistant to TYLCD, and that other measures such as yield traits would need to be considered when selecting varieties for inclusion in further trials.

Yield Measures

A variety of yield measures were collected during the 2006-2007 advanced trials to help determine the appropriateness of the evaluated cultivars for local production needs. Total yield and marketable yield were calculated for each trial (with the exception of Burkina Faso, which only reported total yield). In addition, while no specific guidelines were provided for the collection of fruit characteristics such as fruit size and weight, and yield traits such as fruits per plant, the collection of those data were discussed at the annual planning meeting preceding the 2006-2007 advanced trial and therefore many of the trial managers collected at least some of those data.

Table 4.3 shows yield-related measures for the 11 tomato cultivars included in all advanced trials. (Data from varieties included in only some of the trials can be found in the trial data located in Appendix 2.) The length-to-diameter ratio (L/D ratio) provides a measure of the fruit shape for a given variety – values around 1.0 represent spherical fruits, while values above 1.0 represent long, plum-shaped fruits and values

Table 4.3 – Yield traits in the advanced trials. Superscripts indicate groupings supported by a Tukey HSD test, $p < .05$. FPP: Fruits per plant. L/D: Length/Diameter.

Variety	Total Yield
Industry DR 10403	38.65[a]
Yosra	34.46[ab]
Atak	33.56[ab]
Gempride	32.19[ab]
Bybal	30.07[ab]
Thoriya	29.83[ab]
Realeza	29.67[ab]
Roma VF	28.75[b]
Ponchita	28.18[b]
Chenoa	27.82[b]
Lety F1	23.71[b]

Variety	Weight
Bybal	148.8[a]
Yosra	118.4[ab]
Atak	112.5[abc]
Industry DR 10403	101.1[bc]
Realeza	81.4[bcd]
Chenoa	91.1[bcd]
Gempride	80.2[cd]
Ponchita	80.1[cd]
Roma VF	63.9[d]
Thoriya	58.2[e]
Lety F1	31.1[e]

Variety	FPP
Lety F1	88.6[a]
Gempride	58.8[b]
Industry DR 10403	42.8[bc]
Realeza	46.5[bcd]
Thoriya	37.6[bcde]
Roma VF	39.1[bcde]
Atak	34.7[bcde]
Yosra	30.0[cdef]
Ponchita	28.2[def]
Chenoa	25.9[ef]
Bybal	23.6[f]

Variety	Length
Realeza	60.4[a]
Roma VF	57.0[ab]
Industry DR 10403	55.9[abc]
Thoriya	56.1[abc]
Bybal	54.6[abcd]
Gempride	50.6[bcde]
Yosra	49.8[cde]
Chenoa	49.9[cde]
Atak	48.5[de]
Ponchita	44.2[e]
Lety F1	34.4[f]

Variety	Diameter
Bybal	63.7[a]
Yosra	58.2[ab]
Atak	59.0[ab]
Industry DR 10403	55.6[abc]
Chenoa	55.0[abc]
Gempride	51.9[bcd]
Ponchita	53.7[bcd]
Realeza	47.0[cde]
Thoriya	45.1[de]
Roma VF	39.5[e]
Lety F1	36.8[e]

Variety	L/D Ratio
Atak	0.82
Ponchita	0.82
Yosra	0.86
Bybal	0.86
Chenoa	0.91
Lety F1	0.94
Gempride	0.98
Industry DR 10403	1.01
Thoriya	1.24
Realeza	1.29
Roma VF	1.44

below 1.0 represent flattened, beefsteak types. Several trends are clear in these data. Firstly, Lety F1 clearly falls into its own category as a small, nearly spherical cherry tomato with a very high number of fruits per plant. Of the other varieties, several, such as Roma VF, Thoriya, and Realeza, have a plum-type shape, and tend to have relatively low fruit weight and a moderate number of fruits per plant. Others, such as Industry DR 10403, Gempride, and Chenoa, are spherical to slightly flattened, have moderate fruit weight, and may have relatively high numbers of fruits per plant (though Chenoa does not). Finally, varieties such as Bybal, Yosra, Ponchita and Atak

are flattened spheres and tend to have high fruit weight and low numbers of fruits per plant. Notably, Industry DR 10403 is the only variety to have significantly higher length, diameter, weight and number of fruits per plant than the varieties with the lowest measurements in each of those categories, which may explain its also being the only variety to have a significantly higher total yield than the lowest-yielding varieties.

Farmer Preferences

Many research partners solicited farmer opinions during the trial, either informally or during field days when groups of farmers were brought to see the trials for a more formal evaluation process. In general, farmer response to the cultivars was very positive. Farmers were impressed with the high levels of disease resistance shown by the cultivars, and tended to gravitate towards fruits with firm textures, which hold up better under the rough transport conditions in West Africa. Flavor was also mentioned as a major factor in selection, though farmers in Benin noted that an advantage of less flavorful tomatoes is that they are less appealing to *Helicoverpa armigera* and therefore less likely to be damaged prior to harvest.

Research partners in Baguineda, Mali had farmers rank cultivars in order of preference. From highest to lowest preference, farmers ranked the varieties as follows: Industry DR 10403, HA 3060, HMX 4810, Cheyenne E448, and Gempride. It is not clear if all cultivars were offered for evaluation, or if the five included in the ranking were the only ones evaluated.

It is important to note that farmer opinion, especially when collected in this anecdotal manner, has limited relevance to the potential agronomic or commercial success of a variety. Farmers are likely very familiar with their consumers' tastes, but those tastes

are based on the current landscape of available varieties and do not reflect what consumers might prefer given access to *all* varieties. Furthermore, the possibility of reviving the tomato processing industry in West Africa (see chapter 8) means that consumer preference might have no bearing at all on the success of an introduced variety. Despite all these factors, the collection of farmer opinion is nonetheless informative as it provides an indication of the likely willingness of farmers to adopt the new varieties when they are introduced. If the most disease-resistant and high-yielding varieties were also the least popular among farmers, it would be relevant to consider whether an incentivization program might be necessary to improve adoption rates. In our case, given the high popularity of the most successful varieties, it was clear that preferences would not be a major factor in limiting the adoption of the selected varieties.

Cultivar Selection

Given the high performance of so many materials in the trials, it was difficult for research partners to make selections. Each research partner submitted a list of all cultivars they would like to include in multi-location trials in the following year. Four varieties were requested nearly unanimously, and were therefore selected for all multi-location trials. They were Atak, Bybal, Gempride, and Industry DR 10403. In addition, research partners had the opportunity to select two more cultivars for inclusion in the following year's trial.

Recommendations for Year 3

The 2007-2008 multi-location trials were designed to evaluate selected cultivars in realistic farm conditions and to introduce them to farmers, and it was therefore decided to conduct them on rented farm plots in major tomato production areas. It was

generally agreed that the protocol for the advanced trial had been very successful, but that several aspects needed updating. In particular plot design in the advanced trial was seen as somewhat weak due to the lack of a significant number of plants of Roma VF interspersed among all the trial varieties for better infiltration of virus pressure. The elementary plots were therefore redesigned to include one row of test cultivar surrounded by rows of Roma VF. In addition, low germination rates in a number of the advanced trials called attention to the need for a uniform protocol for the establishment of seedling nurseries.

The following chapter presents the results of the 2007-2008 multi-location trials.

CHAPTER 5

YEAR 3: MULTI-LOCATION TRIAL

Introduction

In 2007-2008 the top-performing TYLCD-resistant tomato cultivars selected during the previous two years' trials were evaluated in multi-location trials throughout West Africa. While similar to advanced trials in plot design and recorded measurements, these trials had several features that were specifically designed to transition selected cultivars from variety trials into farmers' fields. Firstly, the trials were conducted on rented farmland, rather than on research stations, allowing them to function as demonstration plots for the varieties under evaluation. It was expected that farmers viewing the trials would become interested in the new varieties, and that word of mouth would help spread the news about their performance, decreasing the risk that might be associated with adopting those varieties in subsequent seasons. Secondly, the trials were conducted in two locations in each country, preferably within distinct farming communities, to maximize the exposure of the selected cultivars to farmers. When possible, these different farming communities were additionally located in different agroecological zones to determine the extent to which each variety would be well-adapted in multiple environments.

A very significant delay in funding distribution in 2007 unfortunately caused several of the multi-location trials to be canceled, and several more to be planted late. While in previous years the funding from USAID for the NARS partners was routed through AVRDC, in 2007 the funding was instead routed through the Institut du Sahel (INSAH), which waited several months before disbursing the funds. While some of the NARS partners were able to secure advances from their own agencies to cover the

costs of the trials until the funding from USAID arrived, those in Burkina Faso and Senegal were not able to do so, and therefore they did not plant any trials in 2007-2008. The remaining trials were not planted until as late as January of 2008, representing a serious delay from previous years.

Materials and Methods

Plant Materials

Forty initial entries were evaluated throughout West Africa over the course of two rounds of variety trials from 2005-2007. Based on TYLCD resistance, yield, and fruit types, the four highest-performing varieties were selected for inclusion in the 2007-2008 multi-location trial. They were Atak, Bybal, Gempride, and Industry DR 10403. Seeds of these four varieties were requested from their sources, but unfortunately De Ruiter Seeds did not have a sufficient supply of Industry DR 10403 for inclusion in the trials. As a result, Yosra was selected to replace Industry DR 10403 as the fourth variety in the trials. However, since there were not enough seeds of Yosra, it was not evaluated in Mali. In addition, each NARS partner was given the option of including at least two more cultivars in their multilocation trial. Selected varieties included HA 3060, HMX 4810, Lety F1, Ponchita, Realeza, and Thoriya (Table 5.1).

Trial Locations

Though the seven partner countries intended to participate in the trial, Burkina Faso and Senegal were not able to conduct trials due to lack of funding, and therefore trials were only conducted in Benin, Ghana, Mali, Niger, and Togo. In each country, two locations were selected, representing two distinct tomato farming communities (Figure 5.1). If possible, these communities were to be located in distinct agroecological

Table 5.1 – Varieties included in the multi-location trials in Benin (BEN), Ghana (GHA), Mali (MAL), Niger (NIG), and Togo (TOG).

Seed Source	Variety Name	BEN	GHA	MAL	NIG	TOG
De Ruiter Seeds	Bybal	✓	✓	✓	✓	✓
	Lety F1		✓			
	Realeza	✓				
	Thoriya	✓				
Enza Zaden	Atak	✓	✓	✓	✓	✓
	Ponchita			✓	✓	
	Yosra	✓	✓		✓	✓
Harris Moran	HMX 4810			✓		
Hazera	HA 3060					✓
Seminis	Gempride	✓	✓	✓	✓	✓
Tropicasem	Roma VF	✓	✓	✓	✓	✓

zones. Trials were to be conducted on farms, rather than on research stations; in many cases, trial managers hired local farmers to manage the day-to-day maintenance of the trials to ensure that trials would be managed as per local customs. If local farm plots were too small for an entire trial, trials could be split across multiple farms which each farm representing a different block.

Trial Establishment and Maintenance

The trial protocol was again very similar to the previous year's, with a few notable changes to further improve management practices. Detailed instructions were provided for the establishment of seedling nurseries: seeds were to be treated with a fungicide (thioral) and planted at 5cm spacing in seedling trays filled with a pasteurized 1:1:1 mixture of sand, clay and manure. Seedling nurseries were to be watered lightly every day and protected from bright sunlight. Two weeks after emergence seedlings were to be fertilized with a urea solution, and three weeks after emergence watering was to be reduced to prepare seedlings for transplant. Seedlings were to be transplanted four

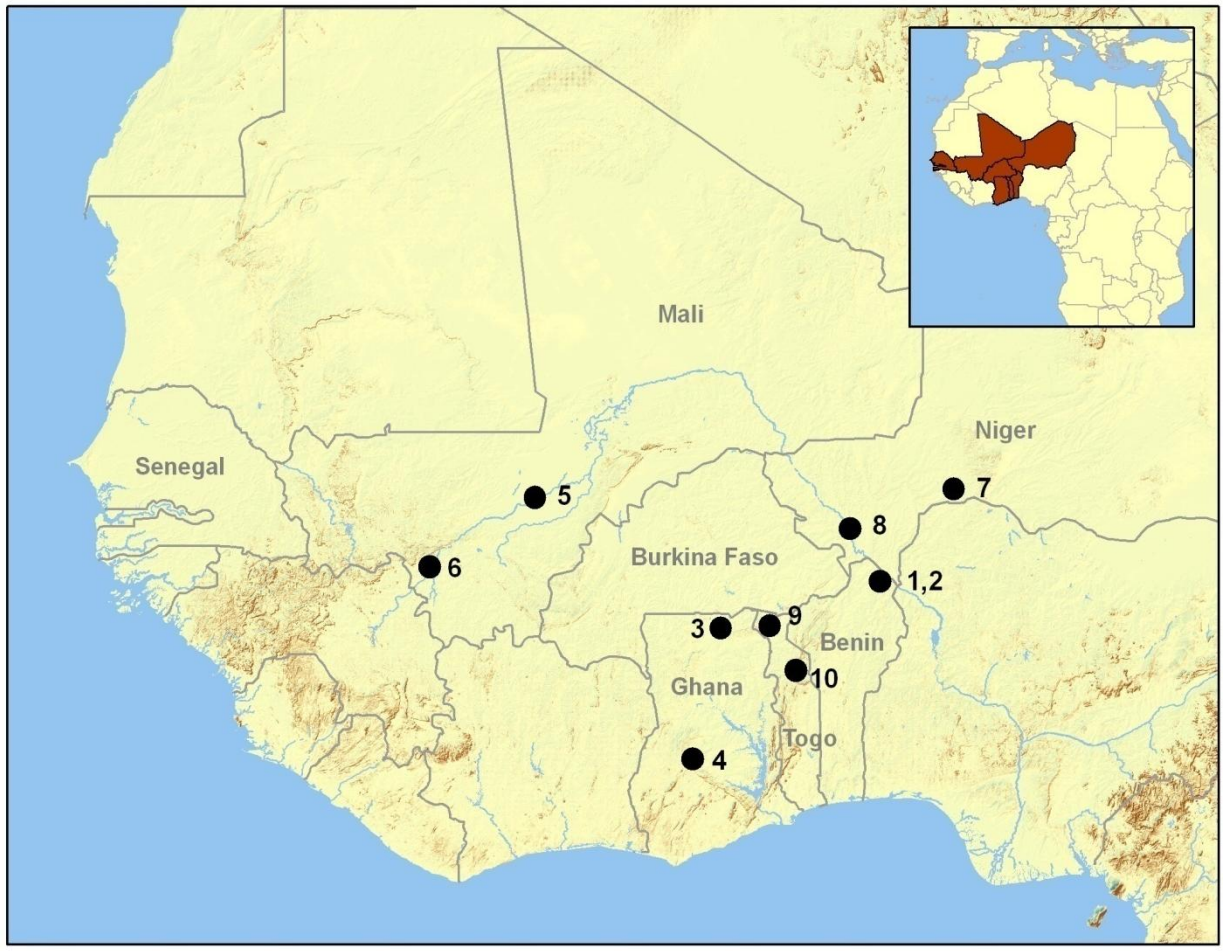

Figure 5.1 – Map of the 2007-2008 multi-location trial sites. 1, 2 – Kargui and Tomboutou, Benin; 3 – Navrongo, Ghana; 4 – Techimantia, Ghana; 5 –Djakorba, Mali; 6 – Sibby, Mali; 7 – Birni N'Konni, Niger; 8 – Kollo, Niger; 9 – Dapaong, Togo; 10 – Kara, Togo.

weeks after emergence in soil up to their first leaves. In addition, a more detailed fertilization schedule for trial plots was provided.

Multi-location trials again entailed a randomized complete block design with three replicates. Elementary plot layout, however, was changed to increase disease pressure and to minimize edge effects: seedlings of varieties under evaluation were planted in single rows of 12 plants surrounded on both sides by spreader rows of 12 plants of Roma VF. Within-row spacing was .5 m and between-row spacing was .6 m, and the total size of each elementary plot was 2 m × 6 m.

Disease severity scoring and yield calculations

Disease scoring was again performed at flowering, fruiting, and first harvest, according to the symptom severity scale described in Chapter 3 (Figure 3.2). Total and commercial yields were also collected as per the previous year's protocol. Collection of yield characteristics from one location per country were formalized in the multi-location trial protocol. Three plants in each elementary plot in the selected location were tagged prior to fruiting, so as to avoid biasing data towards particularly high-yielding plants. The number of fruits per plant and the number of fruits per cluster were to be calculated for each marked plant. In addition, 15 fruits were to be randomly selected from each variety's yield, and those fruits were to be measured for weight in grams, length and diameter in millimeters, and firmness on a subjective scale of 1-3.

Statistical analysis of yield data was again performed using R.

Results

Benin (INRAB)

The multi-location trials in Benin were conducted in Kargui, the site of the previous year's advanced trial, and the village of Tomboutou, approximately 20 km to the east. It actually would have been significantly easier for the research partners from Benin to situate one of the trials further to the south, closer to their offices in Cotonou, but they misunderstood the reasons for choosing multiple locations and chose a second northern site to *avoid* having trials in two distinct agroecological zones. Seeds for both trials were sown on Dec. 4, 2007, and seedlings were transplanted on Jan. 11, 2008.

TYLCD severity in Kargui was moderate, with Roma VF developing a symptom severity score of approximately 3.3 by first harvest, while all other varieties had scores less than 1.0 (Figure 3.2). Farmers in the village described the symptoms of TYLCD as a major problem affecting their tomato production, implying that the moderate pressure is sufficient to have a serious impact on yield. The trial in Tomboutou had somewhat higher disease pressure, though symptom severities were only scored at flowering and fruiting (Figure 3.3). At fruiting, Roma VF had an average TYLCD symptom severity score of 2.8 in Tomboutou, compared with just 1.7 in Kargui. At that timepoint all varieties under evaluation in Tomboutou had scores under 1.0 with the exception of Gempride, which had a symptom severity score of 1.4.

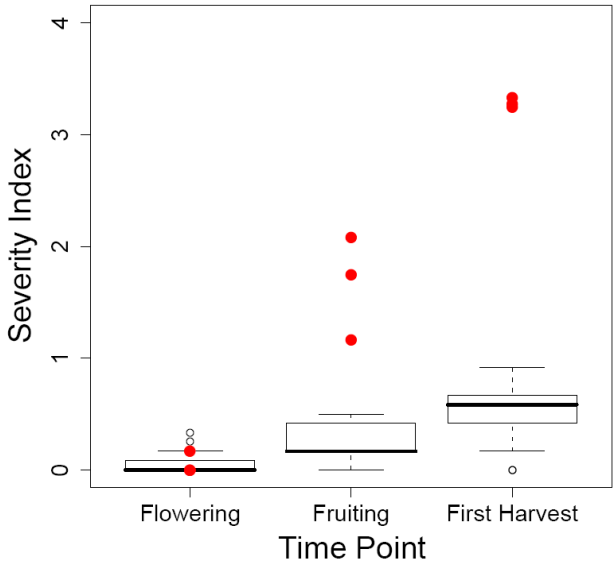

Figure 5.2 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Kargui, Benin. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

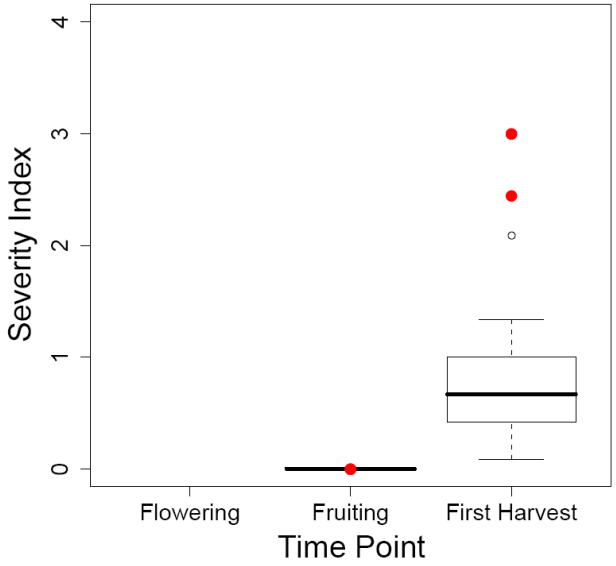

Figure 5.3 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Tomboutou, Benin. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Yields were significantly higher in Kargui than in Tomboutou, possibly due to the higher TYLCD pressure though also possibly due to inconsistent management practices. In Kargui, yields ranged from 31.4 t/ha for Realeza to 11.3 t/ha for Roma VF. Realeza and Thoriya were both found to have significantly higher yields than Roma VF. Marketable yields were only slightly lower than total yields in Kargui, and had the same statistical significance. In Tomboutou, yields ranged from 13.6 t/ha for Atak to just 2.1 t/ha for Roma VF, and no significant differences were found between any of the total or marketable yields.

Aside from fruit size and weight, which will be discussed below, the research partners from Benin also reported instances of blossom end rot and cracking. Roma VF was the only variety that was susceptible to blossom end rot, and Atak was reported to be prone to cracking.

Many farmers in the region visited the trials and were interested in the cultivars under evaluation. They expressed a strong preference for hard fruit, which they said are necessary for shipping. They did express that flavor is the second most important trait to them when selecting tomato varieties. Of the varieties in the trial, Atak appeared to be the favorite among the local villagers in Kargui, while the farmer managing the trial in Tomboutou expressed a preference for Yosra. Several farmers expressed a lack of interest in Gempride because it is a relatively soft-fruited variety.

Ghana (CRI)

The Ghana multi-location trials were held in Navrongo and in Techimanitia. Navrongo, the site of the previous year's advanced trial, is a major tomato growing center in the northern part of the country, near the border with Burkina Faso.

Techimantia is also a major tomato growing region, though tomatoes there tend to be grown during the rainy season. It is located in the much more humid southern region, approximately 70 km northwest of Kumasi.

TYLCD pressure was high and very similar between the two trial locations, though just slightly higher in Techimantia than in Navrongo. In Techimantia Roma VF had an average symptom severity score of approximately 3.7, implying very strong disease pressure (Figure 5.4). Under that pressure Lety F1 scored below 1.0, Yosra scored approximately 1.0, and Atak, Bybal and Gempride scored between 1.0 and 2.0, with Gempride having the highest score. In Navrongo Roma VF had an average score of approximately 3.2, and all other varieties scored in the same ranges as in Techimantia (Figure 5.5).

Yield data were presented for Navrongo, but not for Technimantia. In Techimantia local farmers harvested all of the fruit for themselves since the fruit quality and yield were much higher than that of their own cultivars. This unfortunate turn of events did have a silver lining: farmers were well versed in the different varieties in the trials and were happy to discuss their preferences. Lety F1 was listed as the favored tomato, since it has much higher yield and better flavor than the local cherry tomato. Cherry-type tomatoes are slowly gaining popularity in the region, and though farmers expressed some concern about the size of the market for cherry tomatoes, they mostly agreed that Lety F1 was of sufficient quality to have no marketability issues. In order of preference, the remaining cultivars were ranked as follows: Yosra, Bybal, Atak, Gempride, and Roma VF.

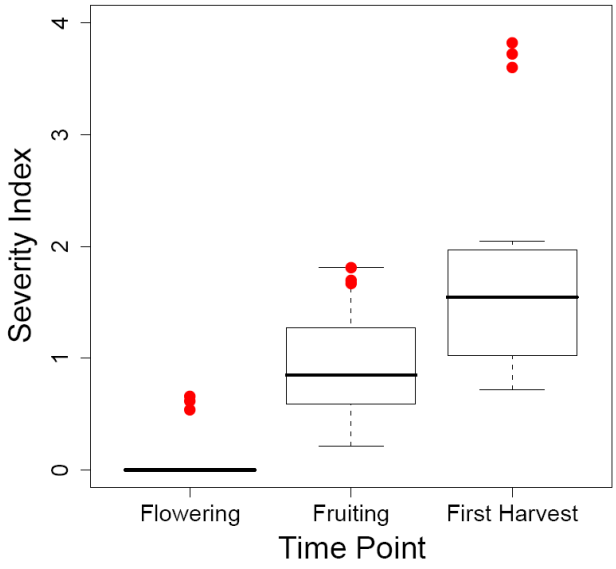

Figure 5.4 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Technimantia, Ghana. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

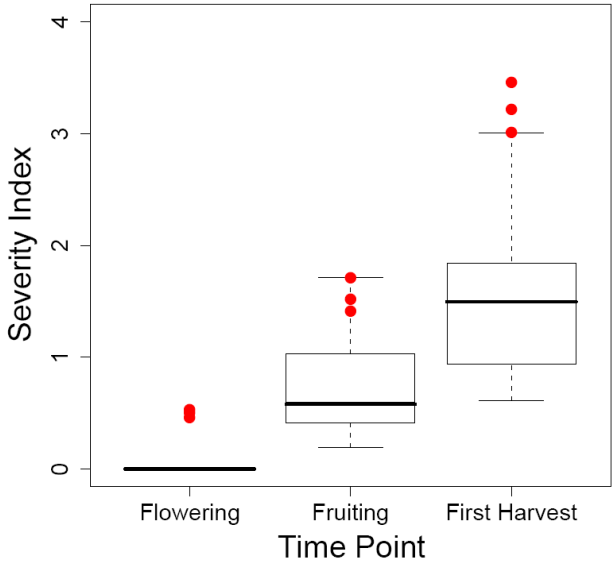

Figure 5.5 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Navrongo, Ghana. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Yields in Navrongo ranged from 44.2 t/ha for Yosra to just 9.9 t/ha for Roma VF. With the exception of Yosra and Bybal, the yields of all varieties were significantly different from the yields of all other varieties. For some of the higher yielding varieties, marketable yields were quite a bit lower, differing from total yields by as much as 8 t/ha. Again, nearly all marketable yields were found to be statistically significantly different from one another – Atak and Gempride were the only two varieties whose marketable yields were not significantly different.

Mali (AVRDC)

Since IER had had difficulties identifying sites with high TYLCD pressure in the previous two years, AVRDC was the only organization to conduct multi-location trials in Mali in 2007-2008. The trials were conducted in Sibby, a town 40 km southwest of Bamako towards the border with Guinea, and in Djakorba, a Millennium Village near Segou, approximately 300 km north of Bamako. While both villages are located within the Sudano-Sehelian climatic zone, Sibby is closer to the Guinean Zone and has much higher humidity than Djakorba, which is nearly in the Sahel.

Red spider mites proved to be a major constraint in both Djakorba and Sibby, eventually destroying both trials and therefore preventing collection of some of the data. In Sibby, only one TYLCD symptom severity time point was collected before the trial was destroyed by the red spider mites. In Djakorba, the red spider mites did not become a serious problem until mid-harvest, allowing for the collection of more data. However, water stress seemed to be a problem in that trial: the empowered mentality of the Millennium Villagers had led many of them to volunteer to manage the trial, and they therefore split it among themselves on a plot-by-plot basis. The result was that each elementary plot was being managed by a different person, and some of those

people were more diligent about watering than others. This problem was eventually discovered and corrected, but during the earlier stages of plant development some plots were severely under-watered and others were not, potentially affecting yields.

In Sibby, TYLCD pressure at flowering was observed to be quite high, with Roma VF having a TYLCD symptom severity score of 1.8, which is high for such an early time point. Most other varieties showed much less susceptibility – HMX 4810 had a score of 0.4, and Atak, Bybal, Gempride and Ponchita remained symptomless. TYLCD pressure was also high in Djakorba, where by first harvest Roma VF had developed a TYLCD symptom severity score of 3.7 (Figure 5.6). At that time point Gempride had a score of 1.8, HMX 4810 had a score of 0.8, and Atak, Bybal and Ponchita remained symptomless. Only two of eight harvests were conducted in Djakorba before red spider mites destroyed the trial, and thus yields were exceedingly low and difficult to

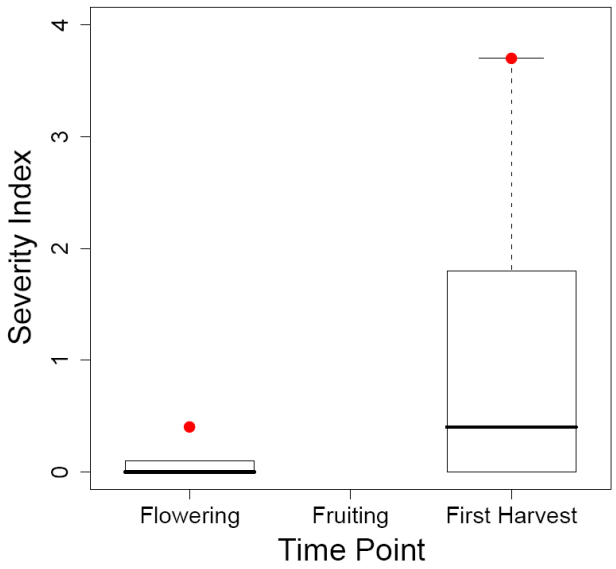

Figure 5.6 – Box and whisker plot of the distribution of TYLCD symptom severity measures at two time points in Djakorba, Mali. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

compare. It is notable, however, that Gempride had a very low yield compared to the other cultivars – this may be because of its higher susceptibility to TYLCD, but it was observed that at least one of the plots containing Gempride had been severely under-watered earlier in the season, so it is difficult to know the cause of Gempride's low yield.

Farmers in Djakorba specifically mentioned a preference for Atak and Bybal, which they liked because of their large fruits. In the year following the trial, excess seeds of HA 3060 and HMX 4810 were distributed to farmers in N'Tonimba, approximately 10 km northwest of Bamako. These farmers have reported receiving a premium price for the fruit of these cultivars, and have expressed strong and repeated interest in gaining access to further seeds.

Niger (INRAN)

Despite a history of low TYLCD pressure, INRAN did conduct multi-location trials in 2007-2008 to introduce farmers to the advantages of modern tomato varieties. Trials were conducted in Kollo, a town approximately 80 km east of Niamey, and in Birni N'Konni, the town where the advanced trial had been situated the previous year. Seeds for the trials were sown extremely late, on Feb. 11, 2008 in Birni N'Konni and on Feb. 13 in Kollo, and transplanting took place on Feb. 19 in Birni N'Konni and on Feb. 24 in Kollo. Harvest spanned from May 27 to June 10 in Birni N'Konni, and from May 28 to June 14 in Kollo.

There was again no TYLCD pressure in the Nigerien trials, and therefore TYLCD symptom severity scores were not reported. Yields were reported, and varied significantly between Birni N'Konni and Kollo. In Kollo the yields were extremely

low, ranging from 2.3 t/ha for Roma VF to just 8.0 t/ha for Bybal, and were not statistically significantly different from one another. No particular constraint was reported to explain the low yields, implying that management practices might have been to blame. Yields in Birni N'Konni, on the other hand, were significantly higher and fell into three groups, with Yosra having the highest yield of 19.6 t/ha, followed by Atak, Bybal, and Ponchita, which were not significantly different from each other and averaged approximately 15 t/ha, and finally followed by Gempride and Roma VF, which were not significantly different from each other and which had yields of 7.9 t/ha and 5.0 t/ha respectively. Marketable yields were primarily less than 1 t/ha lower than total yields, and fell into the same three groups as total yields.

Togo (ITRA)

The Togalese multi-location trials were conducted in Dapaong and Kara. Dapaong, in the far north of the country, was also the site of the previous year's advanced trial. Kara is a town approximately 200 km south of Dapaong – it is also considered to be in northern Togo, and does have a similar Sudano-Sahelian climate to that of Dapaong. Both towns are major centers of tomato production in Togo. Seeds for the trial in Dapaong were sown on Dec. 7, 2007, and seedlings were transplanted on Jan. 4, 2008. TYLCD symptom severity scores were measured on Feb. 18, March 13, and Apr. 2, 2008. Seeds for the trial in Kara were sown on Dec. 8, 2007, and seedlings were transplanted on Jan. 6, 2008. Symptom severity scores were recorded on Feb. 9, March 7, and March 22, 2008.

During the trial, TYLCD symptoms were observed on tomato in farmers' fields surrounding the trial in Dapaong. Nonetheless, TYLCD severity in the trial was scored as only moderate, with Roma VF having a symptom severity score of only 2.8 at first

harvest (Figure 5.7). Furthermore, all cultivars under evaluation developed symptoms to a similar degree, with symptom severity scores ranging from 1.7 for Bybal to 2.3 for Yosra. Similar results were observed in Kara, where Roma VF developed a TYLCD symptom severity score of 3.4, and the scores of the test cultivars ranged from 2.1 for Gempride to 3.3 for Atak (Figure 5.8). There are several possible explanations for the narrow distribution of TYLCD symptom severity scores observed in these trials. Very strong bacterial wilt symptoms were observed at the trial in Kara, and those symptoms could have been mistaken for TYLCD, giving the impression that all varieties were essentially equally susceptible to the virus. While bacterial wilt was not specifically observed in Dapaong, there is precedent for non-begomovirus-associated diseases being associated with TYLCD in Togo, and therefore it is plausible that symptom severity scoring was not accurately reflective of TYLCD symptom severity. An alternative explanation is that none of the varieties in the trials were strongly resistant to the newly discovered Tomato Leaf Curl Togo Virus (ToLCTgV), which may be the dominant tomato-infecting begomovirus in Togo, though more research will need to be conducted to confirm this. ToLCTgV is associated with a novel DNA-β that may be responsible for "breaking" the resistance of the TYLCD-resistant tomato cultivars included in the trial. For this to be the case, it would also need to be true that ToLCTgV and its DNA-β are only moderately virulent, since even Roma VF did not develop strong symptoms in either trial.

Yields in the Togalese trials were relatively low. In Dapaong, total yields ranged from 13.3 t/ha for Atak to 3.2 t/ha for HA 3060. Marketable yields were consistently exactly 6.4% lower than total yields, implying that trial managers estimated marketable yields rather than calculating them directly. No significant differences in yield were found between any cultivars in the trial. In Kara, yields ranged from 28.9 t/ha for Thoriya to

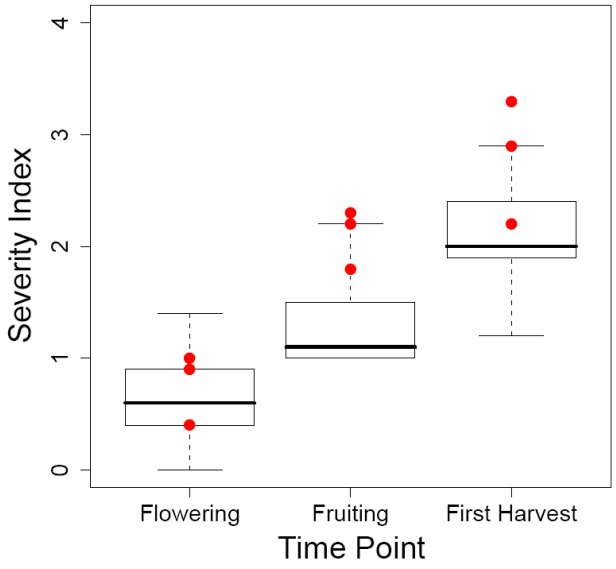

Figure 5.7 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Dapaong, Togo. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

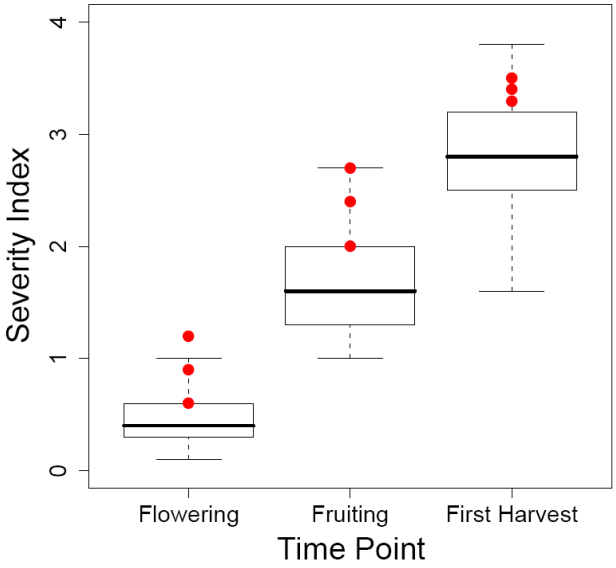

Figure 5.8 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Kara, Togo. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

10.0 t/ha for Atak. Marketable yields were 21.8% lower than total yields in the trial. Thoriya was found to have a significantly higher yield than four other varieties: Roma VF, Yosra, Bybal, and Atak.

Farmers in Kara visited the trial and expressed a general preference for Atak and Bybal over both the other cultivars in the trial and their locally popular cultivars.

Discussion

The 2007-2008 multi-location trial was the third in a series of variety trials aimed at identifying tomato cultivars suitable for West Africa. While the previous two years' preliminary screening and advanced trials were designed to differentiate between tomato cultivars based on their resistance to TYLCV and their performance in the production environment of West Africa, the 2007-2008 multi-location trial had two different goals: to confirm the performance of the selected cultivars in the less forgiving environment of farmers' fields, and to publicize the varieties to producers in many regions throughout West Africa.

Interestingly, the performance of the selected cultivars did suffer in farmers' fields. The cultivars in the multi-location trials showed more TYLCD symptoms than they had in previous years on research stations under equivalent disease pressure, and had lower yields due to both lower fruit set and to the production of smaller, lighter fruits. Nonetheless, farmers who witnessed the trials were uniformly enthusiastic about the new cultivars and many expressed interest in gaining access to seeds.

TYLCD resistance and cultivar performance

Table 5.2 compares the average performance of the selected cultivars in the 2007-2008 multi-location trials with the performance of the same cultivars in the 2006-2007 advanced trials. The table illustrates the performance advantage of all selected cultivars over Roma VF. In both the advanced trials and the multi-location trials, all selected cultivars had lower average TYLCD symptom severities and higher average yields than Roma VF, demonstrating lower levels of TYLCD susceptibility and greater yield potential. It is notable that average TYLCD symptom severity on Roma VF did not change significantly between year 2 and year 3, implying that there was no change in overall TYLCD pressure in the region. However, the cultivars under evaluation did show approximately two-fold increases in TYLCD symptom severity and two-fold decreases in yield in the switch from the advanced trials to the multi-location trials. This is likely a reflection of the change in environment – while the advanced trials were conducted on research stations with relatively controlled environments and relatively advanced management practices, the multi-location trials were conducted on farms with uncharacterized soils and unknown local disease pressures. While the change to a less-controlled environment had a significant impact on the performance of the selected cultivars, it had an even greater impact on the performance of Roma VF, which saw a four-fold decrease in yield for the same transition. Thus, the selected varieties offer not only disease resistance and increased yield potential, but also stochastic dominance over the regionally popular TYLCD-susceptible tomato varieties. In other words, while performance might vary from one environment to the next, the selected TYLCD-resistant varieties consistently outperform their TYLCD-susceptible counterparts. This is crucial in the heterogeneous, often degraded environments of West Africa where farmers have limited access to inputs and therefore limited control over their environments. By

offering consistently higher yield potential in all environments, the selected varieties promise farmers more predictable returns, and thus more consistent livelihoods.

Table 5.2 – Comparison of the performance of selected cultivars in the advanced trial (AT) of Year 2 (2006-2007) and the multi-location trial (MLT) of Year 3 (2007-2008). Changes in symptom severity (SS) scores and total yields are indicated.

Variety	Year 2 - AT		Year 3 - MLT		Change	
	SS	Yield	SS	Yield	SS	Yield
Atak	0.85	33.56	1.82	16.58	+113%	-51%
Bybal	0.86	30.07	1.65	14.54	+91%	-52%
Gempride	1.06	32.19	1.70	12.48	+61%	-61%
Yosra	1.25	34.46	1.51	16.12	+21%	-53%
Roma VF	3.34	28.75	3.29	6.90	-2%	-76%

Next steps

In all trial locations visiting farmers expressed interest in the cultivars evaluated in the multi-location trials. Atak, Bybal, Lety F1 and Yosra were mentioned frequently, and Gempride was the only variety farmers were not interested in, due to the softness of its fruit. As a result, efforts are underway to introduce those cultivars into seed distribution channels in West Africa. This process is discussed at length in Chapter 8.

CHAPTER 6

YEAR 2: PRELIMINARY SCREENING TRIAL (GROUP 2)

Introduction

During the 2005-2006 TYLCD-resistance trials, several seed companies and public breeders continued to send putatively TYLCD-resistant materials for evaluation. As a result, a second preliminary screening trial was conducted during the 2006-2007 growing seasons to evaluate materials that had been received over the course of the previous year.

Materials and Methods

Plant Materials

A total of 28 tomato cultivars were selected by seed companies and public breeders for inclusion in the 2006-2007 preliminary screening trial. Aside from putative TYLCD resistance, these cultivars were not selected for any shared traits. Table 6.1 lists all included materials and the organization that provided them.

In addition to the 28 tomato cultivars, 25 breeding lines were received, 18 from the tomato breeding program at AVRDC in Taiwan and 7 from the tomato breeding program at the University of Florida. The U. Florida materials used *S. chilense* LA 1932, LA 1969, and LA 2779 as resistance sources. Seeds of the lines from both breeding programs were received in small quantities, and therefore were only trialed in the AVRDC trial at Samanko. Results for these materials will not be discussed in this chapter, but data are available in Appendix 2. Roma VF was again used as the susceptible check variety in the trial.

Table 6.1 – Tomato varieties included in the 2006-2007 preliminary screening trial for resistance to TYLCD.

Seed Source	Variety Name
DeRuiter Seeds	Athyla F1
	Dennolino F1
	DRW 7215 F1
	Industry DR 10401
	Porfyra F1
	Valor F1
Enza Zaden	Aegean
	Espadilha
	Hamoud Mumyes / E 26 31998
	Sensei
	Setcopa
Gentropic	Llanero
Hazera	HA 3019
	HA 3074
Israel ARO - Volcani Center	F1 1494
Nunhems	BWTH CO03
	BWTH CO12
	BWTH CO17
	NUN 5025 TO
Seminis	Gem Pack
	Gem Pear
Seminis - India	Mrutunjanya
SOLI Industries	F1 641
	F1 Floradida 495
	F1 Veuona 483
Syngenta	Nirouz TH 99806
Takii	MT 158
Tropicasem	F1 Savana
	Roma VF

Trial Locations

Trials were conducted concurrently with the 2006-2007 advanced trials in eleven locations in seven countries throughout West Africa (Figure 6.1). Participating agencies were INRAB (Benin), INERA (Burkina Faso), CRI (Ghana), IER (Mali), INRAN (Niger), CDH/ISRA (Senegal), and ITRA (Togo), as well as AVRDC. Each agency chose one site for the establishment of a trial, except for IER, which as a partner on the management of the project chose four sites for trials. All preliminary trials were conducted in the Sudano-Sahelian climatic zone in the same locations as the concurrent advanced trials, with the exception of the trial in Ghana, which was conducted on the same site as the preliminary trial of the previous year in southern Ghana. Figure 6.1 shows the locations of all eleven preliminary trials conducted during the 2006-2007 growing season.

Trial Establishment and Management

Suggested management practices for the 2006-2007 preliminary screening trial matched those of the advanced trial conducted in the same locations at the same time. In particular these included specific practices related to site selection, seedling nursery establishment, and field management. To minimize the impacts of some of the most devastating diseases observed in the preliminary trials of the previous year, the protocol specified that sites must be known to be free of soil-borne illnesses such as root-knot nematodes, *Fusarium oxysporum* f.sp. *lycoperisci*, and *Ralstonia solanacearum*. Seedling nurseries were to be established as per the protocols of the NARS, but the trialing protocol specified that healthy seedlings would be transplanted three to four weeks after germination. Fertilization practices were also specified: both mineral fertilizers (i.e. NPK) and organic matter (farmyard manure or compost) were to be incorporated into the soil in advance of transplanting, and top dressing with NPK

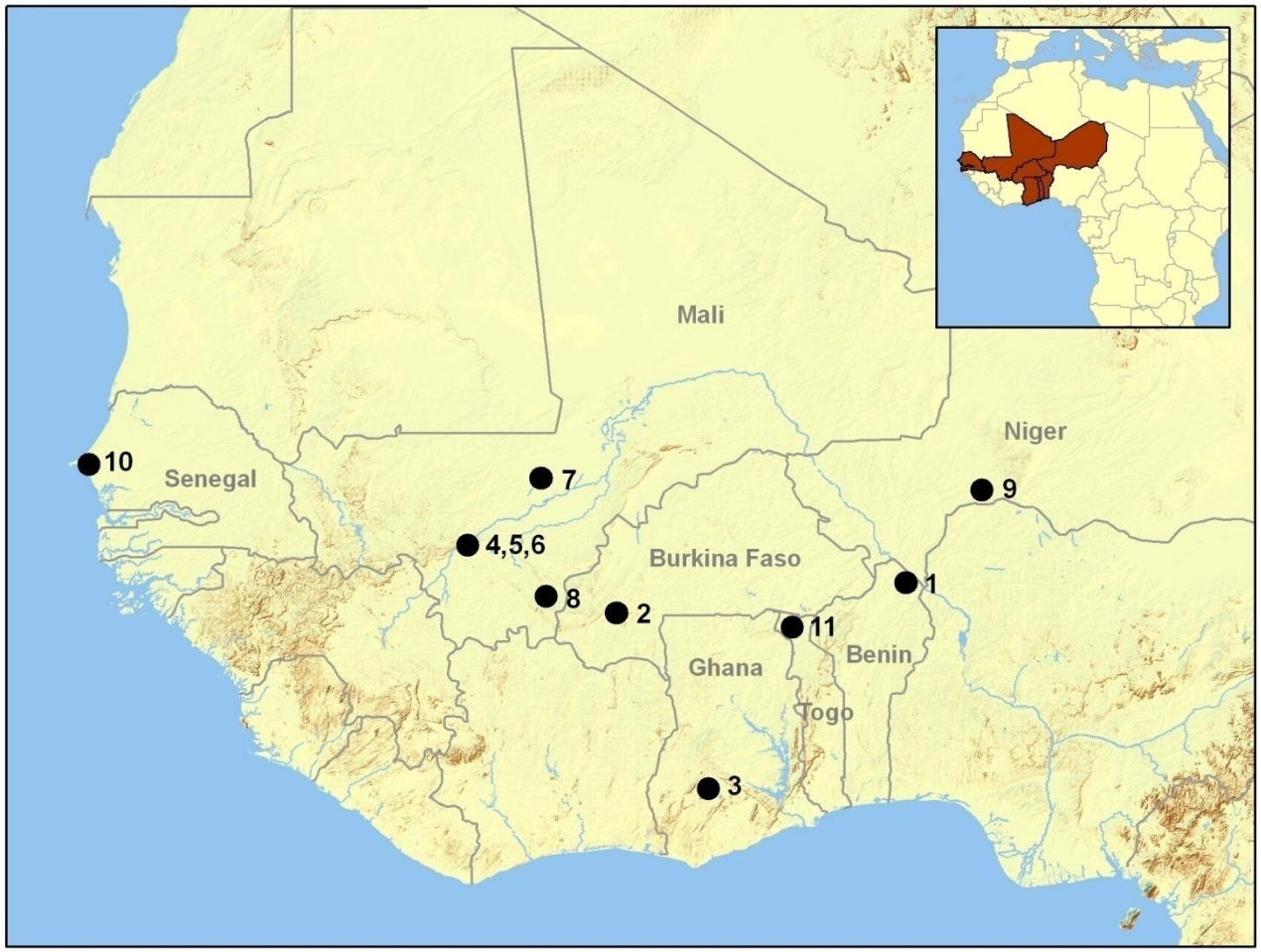

Figure 6.1 – Map of the 2006-2007 preliminary trial locations. 1 – Kargui, Benin; 2 – Bobo Dioulasso, Burkina Faso; 3 – Kumasi, Ghana; 4, 5, and 6 – Baguineda, Samanko and Sotuba, Mali; 7 – Niono, Mali; 8 – Sikasso, Mali; 9 – Birni N'Konni, Niger; 10 – Rufisque, Senegal; 11 – Dapaong, Togo.

was recommended during the growing season. Finally, the protocol specified that *Helicoverpa armigera* and other insect pests would be controlled by regular applications of selective pesticides that did not target whiteflies, and that weeds would be controlled regularly.

Elementary plot layout was again 26 plants per plot laid out in two rows of 13 plants with a spacing of .6 m between rows and .5 m within rows. Elementary plots were laid out in a grid of any dimensions that fit the available space in each location. Empty elementary plots were planted with extra plants of Roma VF to increase incident virus pressure.

Disease severity scoring and yield calculations

Disease scoring was done at three time points during the growing season (flowering, fruiting, and first harvest) and according to the same symptom severity scale used in the 2005-2006 preliminary screening trial (Illustration 3.2). In addition, total and marketable yields were calculated. Since the trial did not involve replication, yields were not statistically analyzed.

Results

Benin (INRAB)

The preliminary trial in Benin was conducted in the village of Kargui, in the far north of the country near the city of Malanville and the borders with Burkina Faso and Niger. Seeds were sown on Nov. 20, 2006, and seedlings were transplanted on Dec. 18. First harvest took place on March 15, 2007, with the final harvest taking place April 20.

As in the advanced trial of the same year, disease pressure in Benin was relatively mild. The vast majority of the tested cultivars remained symptomless at first harvest (Figure 6.2). Roma VF had the highest symptom severity score of 3.5, and 6 other cultivars developed symptoms: Gem Pack, HA 3074, Mrutunjanya, Valor F1, F1 Veuona 483, and F1 Floradida 495. Yields in the preliminary trial ranged from a high of 37.3 t/ha for Llanero to 10.0 t/ha for Roma VF. Marketable yields were very close to total yields, being on average just 1.7% lower. This may reflect the very good management practices of the Beninoise trial managers (as described in Chapter 4).

Burkina Faso (INERA)

The 2006-2007 preliminary trial in Burkina Faso was conducted in the Kou Valley near Bobo Dioulasso, and experienced very high TYLCD pressure. Seeds for the trial were sown on Nov. 22, 2006 and transplanted on Dec. 22. As in the advanced trial in

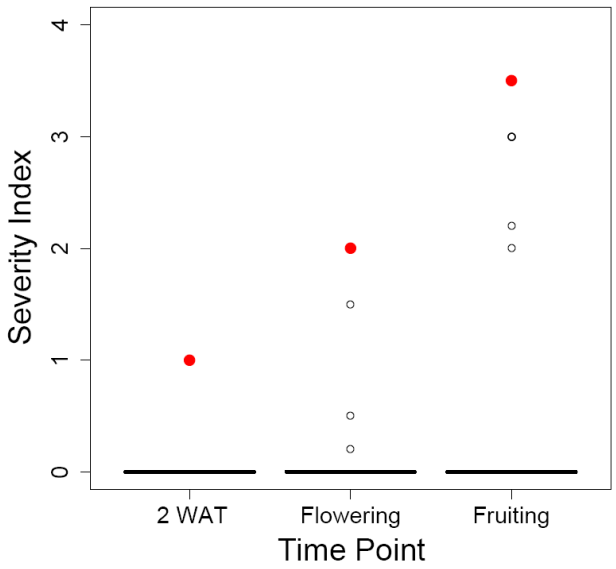

Figure 6.2 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Kargui, Benin. Red dots represent the TYLCD symptom severity scores of Roma VF.

the same year, whitefly populations were very high in the trial, and TYLCD symptoms were observed to be very high in neighboring fields early on in the trial. However, root-knot nematodes and bacterial wilt were also observed, and appeared to be overwhelming the TYLCD symptoms at earlier stages of the trial.

The TYLCD symptom severity plot for the trial shows a steady increase in virus pressure over the course of the season, with Roma VF developing a symptom severity score of 3.0 by fruiting and 4.0 by first harvest (Figure 6.3). Cultivars in the trial showed a wide range of responses, from strong resistance to nearly complete susceptibility. Nun 5025 TO was the only variety to score below 1.0, and Dennolino F1, F1 1494, DRW 7215 F1, and Sensei scored below 2.0. Yields in the trial were extremely low, ranging from 1.3 t/ha for BWTH CO03 and BWTH CO17 to 11.9 t/ha for Gem Pear. There were additionally two outliers that might have had very high yields, or might have been mistyped by the project technicians: F1 Savana had a yield

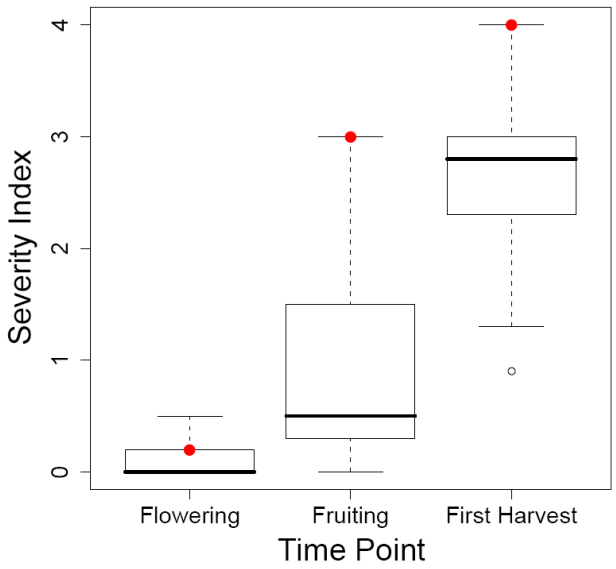

Figure 6.3 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in the Kou Valley, Burkina Faso. Red dots represent the TYLCD symptom severity scores of Roma VF.

of 20.1 t/ha, and Aegean had a score of 50.2 t/ha. Marketable yields were on average 0.6 t/ha lower than total yields; however, due to the very low total yields, this averaged out to a 22% loss.

Interestingly, the most resistant cultivars in the 2006-2007 preliminary trial in Burkina Faso showed stronger disease resistance than the resistant cultivars in the advanced trial conducted that year in the same location. This may imply that the new batch of materials under evaluation in 2006-2007 had some higher-performing varieties than those evaluated during the preliminary trial of 2005-2006. Alternatively, the presence of a disease pressure gradient across the blocks of the advanced trial, as described in Chapter 4, raises the possibility that the preliminary trial was situated at the low end of this pressure gradient. However, the yields in the preliminary trial were much lower than those in the advanced trial, making that possibility less likely.

Ghana (CRI)

The 2006-2007 preliminary trial in Ghana was conducted on the same site as the previous year's preliminary trial in Kumasi, towards the southern end of the country. As in the previous year, the trial was damaged significantly by a wilt disease, preventing the collection of data. However, the disease did not develop significantly until soon before the first harvest, and therefore two TYLCD symptom severity scorings were conducted before the trial was destroyed.

TYLCD pressure in the trial was quite high, with Roma VF showing a symptom severity score of 3.6 by fruiting (Figure 6.4). However, the wilt disease may have been misdiagnosed as TYLCD at first, as the lowest symptom severity score at fruiting was 1.4 (Industry DR 10401). Three varieties scored below 2.0 (Industry DR 10401,

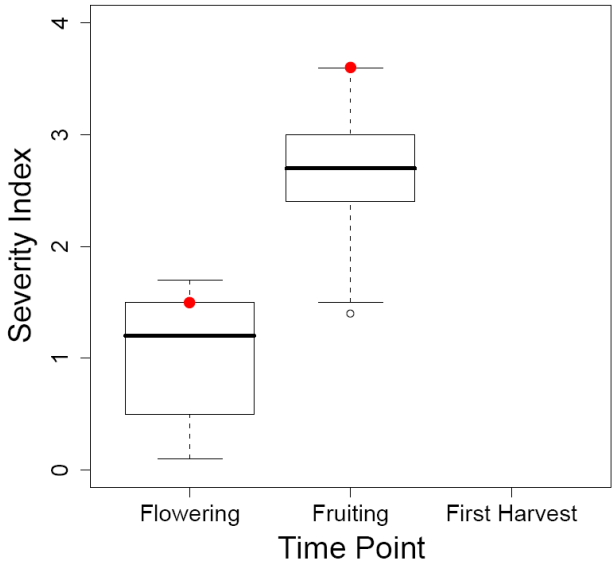

Figure 6.4 – Box and whisker plot of the distribution of TYLCD symptom severity measures at two time points in Kumasi, Ghana. Red dots represent the TYLCD symptom severity scores of Roma VF.

Dennolino F1, and DRW 7215 F1) and three more scored 2.0 (F1 1494, F1 Savana, and Gem Pack).

Yields were not calculated as the trial was destroyed by a wilt prior to the first harvest.

Mali (IER) – Baguineda

As described in Chapter 4, three of the four trials conducted by IER in 2006-2007, located in Niono, Sikasso, and Sotuba, had no incident TYLCD pressure and therefore data were not shared. The trial in Baguineda, in contrast, did experience significant disease pressure, with Roma VF developing a TYLCD symptom severity score of 4.0 by first harvest (Figure 6.5). Three varieties remained symptomless during the trial in Baguineda: F1 1494, F1 Savana, and Sensei. An additional 8 varieties received

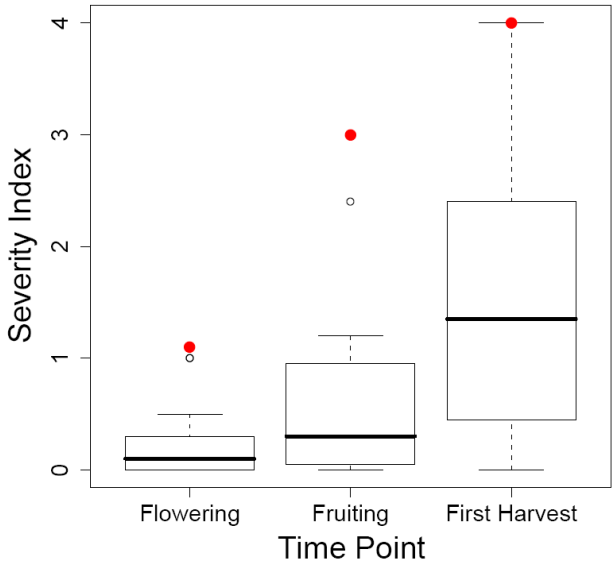

Figure 6.5 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Baguineda, Mali. Red dots represent the TYLCD symptom severity scores of Roma VF.

symptom severity scores of less than 1.0: Espadilha, Dennolino F1, F1 Floradida 495, Porfyra F1, Athyla F1, DRW 7215 F1, Aegean and Setcopa.

Yields were calculated in the trial, however the seeds of four varieties had such low germination rates that an insufficient number of plants was available for calculating yield: BWTH CO17, Llareno, MT 158, and Sensei. This highlighted the need for the development of a protocol for the establishment of seedling nurseries for the subsequent year. The yields that were calculated ranged from 2.1 t/ha for Nirouz TH 99806 to 33.6 t/ha for F1 Savana, with Roma VF having a yield of 9.8 t/ha. Marketable yields were very close to total yields, typically within less than 1 t/ha.

Mali (AVRDC) – Samanko

The 2006-2007 preliminary trial in Samanko included 53 cultivars and breeding lines. Symptom severity scores were only collected at two time points, fruiting and first harvest, but these scores were sufficient for differentiating between resistant and susceptible cultivars. The trial experienced moderate disease pressure, with Roma VF developing a TYLCD symptom severity score of 3.2 by first harvest (Figure 6.6). This is similar to the results seen with the concurrent advanced trial. Two varieties, Athyla F1 and Setcopa, were symptomless at the end of the trial, and 8 varieties had scores of less than 1.0: Dennolino F1, Espadilha, F1 1494, Hamoud Mumyes, Sensei, DRW 7215 F1, Nirouz TH 99806, and F1 Floradida 495. In addition, two breeding lines from University of Florida and one from AVRDC received scores of than 1.0.

Yields were again quite high, ranging from 49.4 t/ha for HA 3019 to 22.2 for Athyla F1. However, marketable yields were quite low, likely due to the development of

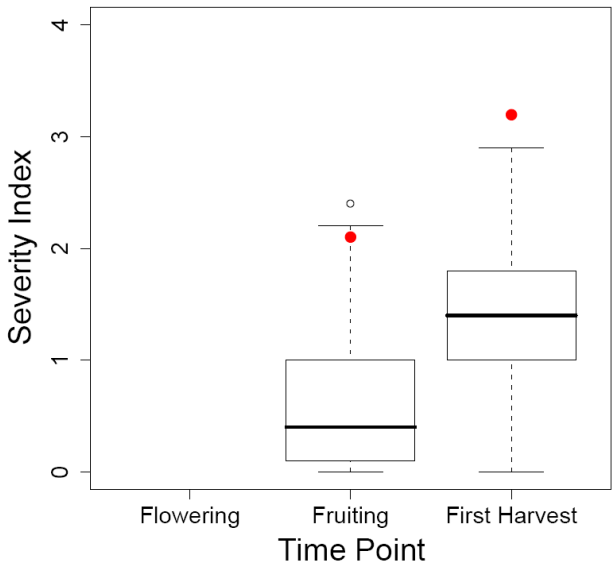

Figure 6.6 – Box and whisker plot of the distribution of TYLCD symptom severity measures at two time points in Samanko, Mali. Red dots represent the TYLCD symptom severity scores of Roma VF.

blossom end rot following a blockage of the drip irrigation system, as described in Chapter 4. Marketable yields ranged from 31.5 t/ha for DRW 7215 F1 to 5.0 t/ha for F1 Floradida 495, with the average loss being 41% of total yield.

Niger (INRAN)

The INRAN preliminary trial in the 2006-2007 growing season was conducted concurrently with the advanced trial in Birni-N'Konni in southern Niger approximately 350 km east of Niamey and less than 10 km from the border with Nigeria. Seeds for the trial were sown on Nov. 16, 2006, and transplanted Dec. 23.

While whitefly populations were observed to be moderately high at the Nigerien advanced trial, TYLCD pressure was very low and no significant symptom levels developed during the course of the trial. Total yields were calculated and had a very significant range, from 49.0 t/ha for F1 1494 to 0.8 t/ha for Mrutunjanya. Notably, the advanced trial did not show any exceedingly low yields, ranging from 58.4 t/ha to 33.0 t/ha. There was no obvious explanation for the wide range in yields in the preliminary trial.

Senegal (CDH/ISRA)

The Senegalese preliminary trial was conducted concurrently with the advanced trial at the Sangalkam research station in Rufisque, about 40 km east of Dakar. Seeds were sown somewhat later than those of the advanced trial, on Nov. 23, 2006, and seedlings were transplanted on Dec. 26. First harvest was on March 3, 2007, and final harvest took place on Apr. 19.

Interestingly, disease pressure in the preliminary trial was quite a bit lower than in the advanced trial, with Roma VF developing a symptom severity score of only 2.9 by first harvest, and with the local susceptible variety Xina developing a score of only 2.1 (Figure 6.7). In contrast, in the advanced trial Roma VF and Xina developed scores of 3.7 and 4.0, respectively. Yields in the preliminary trial ranged from 65.9 t/ha for HMX 4810 to 10.6 t/ha for Espadilha. Marketable yields were on average 40% lower than total yields, with HMX 4810 and Espadilha again showing the maximum and minimum values, respectively.

Togo (ITRA)

In 2006-2007 the Togalese preliminary trial was conducted at the Tantiégou research station in the northern city of Dapaong, near the border with Burkina Faso. As with the advanced trial, TYLCD symptoms may have been overwhelmed by another disease,

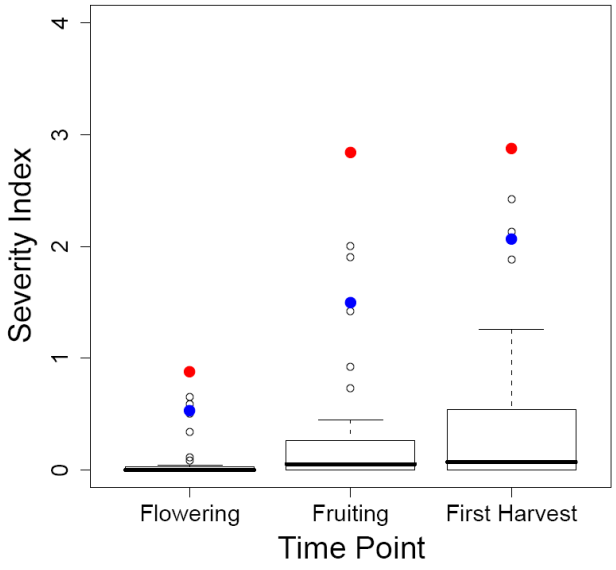

Figure 6.7 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Rufisque, Senegal. Red dots represent the TYLCD symptom severity scores of Roma VF, and blue dots represent the TYLCD symptom severity of locally popular variety Xina.

possibly early blight. In addition, in mid-February it was discovered that trial technicians had been spraying pyrethroid insecticides to control insect populations during the trial, thereby inadvertently reducing whitefly populations and the spread of tomato-infecting begomoviruses. As a result of these two occurrences, symptom severity scores were very low, and Roma VF was not scored as the most susceptible variety (Figure 6.8). BWTH CO03 and F1 1494 shared the highest score of 2.0 at first harvest, while Dennolino, Espadilha, Hamoud Mumyes and Setcopa remained symptomless. Roma VF, in constrast, received a score of 1.4. Yields were reported, and ranged from 31.9 t/ha for Industry DR 10401 to 9.7 t/ha for Hamoud Mumyes. Marketable yields were, on average, about 1.6 t/ha lower than total yields.

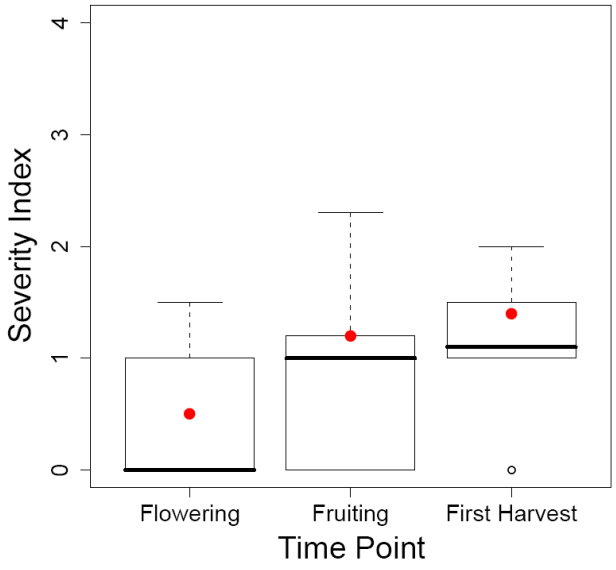

Figure 6.8 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Dapaong, Togo. Red dots represent the TYLCD symptom severity scores of Roma VF.

Discussion

TYLCD resistance and cultivar selection

When compared with the previous year's preliminary trial, the preliminary screening trial of 2006-2007 more consistently exposed cultivars under evaluation to TYLCD and limited exposure to other regionally important diseases including Fusarium wilt, bacterial wilt, and root knot nematodes. This was primarily due to its association with the 2006-2007 advanced trial, which had enhanced management protocols dealing with site selection, fertilization, and pest control to prevent TYLCD symptoms from being masked by other diseases.

Figure 6.9 shows the TYLCD symptom severity score distributions at first harvest for all trial sites. A number of different conditions can be observed. For instance, Benin and Senegal showed very weak TYLCD pressure, with Roma VF nonetheless receiving one of the highest symptom severity scores. The trials in Baguineda and Samanko, Mali, in contrast, experienced moderate disease pressure, with Roma VF showing strong disease symptoms but other varieties in the trial remaining symptomless at first harvest. Finally, the trials in Burkina Faso and Ghana experienced very strong TYLCD pressure and possibly other diseases as well, with Roma VF showing strong disease symptoms and the most resistant cultivars receiving symptom severity scores of approximately 1.0. The trial in Togo was the only trial to yield questionable results, with symptom severities falling into a very small range and with Roma VF receiving a relatively low TYLCD symptom severity score as compared with the highest scorers in the trial.

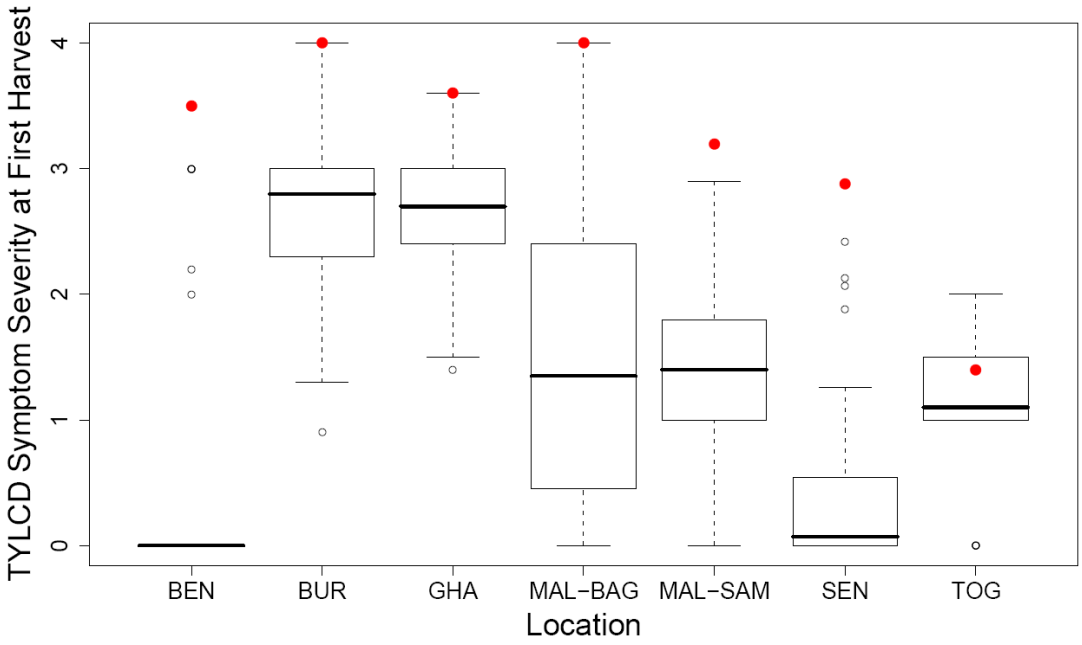

Figure 6.9 – Box and whisker plot of the distribution of TYLCD symptom severity scores at first harvest in each trial location. Red dots represent the variety Roma VF. Locations include Benin (BEN), Burkina Faso (BUR), Ghana (GHA), Baguineda, Mali (MAL-BAG), Samanko, Mali (MAL-SAM), Senegal (SEN), and Togo (TOG). Note that the data shown for Ghana represent TYLCD symptom severity scores at fruiting since no data were collected at first harvest.

Yields could not be statistically analyzed across trials because yield distributions varied very significantly between trial sites. Figure 6.10 shows yield distributions for each trial site, with notches representing what amounts to an approximate 95% confidence interval of the median.

Selections were again made based on votes by all participating NARS partners. Ten varieties were selected for the following year's advanced trial, as shown in Table 6.2.

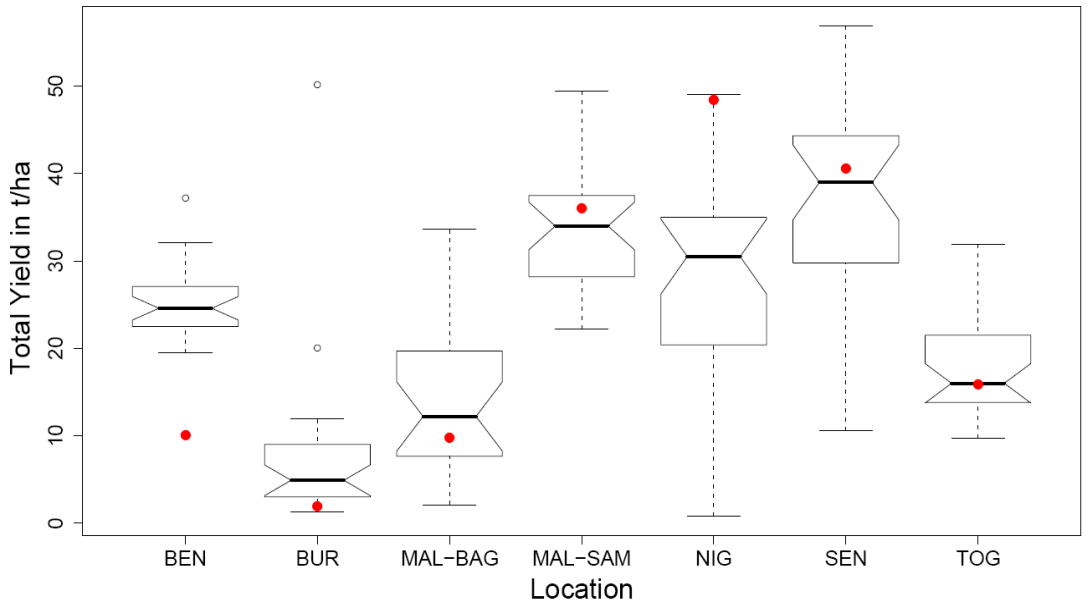

Figure 6.10 – Notched box and whisker plot of the distribution of total yields of all cultivars under evaluation in each trial location. Red dots represent the variety Roma VF. Locations include Benin (BEN), Burkina Faso (BUR), Ghana (GHA), Baguineda, Mali (MAL-BAG), Samanko, Mali (MAL-SAM), Senegal (SEN), and Togo (TOG).

Table 6.2 – Performance of putatively TYLCD-resistant tomato cultivars in the 2006-2007 preliminary screening trials. Numbers represent average TYLCD symptom severity scores at fruiting. For each trial, colors are allocated on a linear scale with yellow representing the lowest symptom severity score and blue representing the highest. Selections for the following year's advanced trial are marked with a check mark in the Selection column. (cont'd on next page)

VARIETY NAME	BEN	BUR	GHA	MAL-BAG	MAL-SAM	SEN	Selection	TOG
Aegean	0	2.3	3	0.8	1	0	✓	1.8
Athyla F1	0	2.7	2.6	0.7	0	0	✓	1.7
BWTH CO03	0	3	2.6	1.3	1.4	1.88		2
Bwth CO12	0	3.4	2.5		2.9	2.42		1
Bwth CO17	0	3.5	2.8	3.5	1.5	2.13		1
Dennolino F1	0	1.3	1.5	0.2	0.1	0	✓	0
DRW 7215 F1	0	1.4	1.5	0.7	0.7	0	✓	1
Espadilha	0	2.8	3.5	0.1	0.5	0	✓	0
F1 1494	0	1.3	2	0	0.5	0		2
F1 641	0	3	2.5	1.9	1.4	0.91		1
F1 Floradida 495	2	3	3	0.2	0.9	0		1.6
F1 Savana	0	2.3	2	0	1.8	0		1.5
F1 Veuona 483	2.2	3	2.6	2.3	2.8	0.41		1.3
Gem Pack	3	3	2	3	2.6	0.54		1.1

Table 6.2 (cont'd) – Location headings are: BEN – Benin; BUR – Burkina Faso; MAL-BAG – Baguineda, Mali; MAL-SAM – Samanko, Mali; SEN – Senegal; TOG – Togo.

VARIETY NAME	BEN	BUR	GHA	MAL-BAG	MAL-SAM	SEN	Selection	TOG
Gem Pear	0	2.8	2.3	3.1	1.6	0.46		1.1
HA 3019	0	2.7	2.9	1.2	1.3	0		1.1
HA 3074	3	2.8	2.7	1.4	1.4	0		1.5
Hamoud Mumyes	0	2.7			0.5	0	✓	0
Industry DR 10401	0	2.9	1.4	2.4	1.4	0.3	✓	1
Llanero	0	3	2.9	3	2	0		1
Mrutunjanya	3	3	2.5	2.3	1.6	0.76		1.5
MT 158	0	2.9	3	1	2.4	0		1.1
Nirouz TH 99806	0	2.8	3.1	3	0.8	0.11		1
NUN 5025 TO	0	0.9	2.7	1.8	1.5	0.07		1.4
Porfyra F1	0	2.2	3.5	0.2	1.2	0	✓	1
Roma VF	3.5	4	3.6	4	3.2	2.88		1.4
Sensei	0	1.4	2.4	0	0.5	0.03	✓	1
Setcopa	0	2.3	3.4	0.8	0	0	✓	0
Valor F1	3	2.9	2.7	2.4	2	0.3		1

The next chapter presents the results of the 2007-2008 advanced trials evaluating the varieties selected in the 2006-2007 preliminary trial.

CHAPTER 7

YEAR 3: ADVANCED TRIAL (GROUP 2)

Introduction

In 2007-2008 an advanced trial was conducted to further evaluate the TYLCD-resistant materials selected in the 2006-2007 preliminary screening trial. Like the advanced trial of 2006-2007, this trial used a replicated design to more fully differentiate between cultivars based on disease resistance, yield, and fruit characteristics. However, several constraints particular to the 2007-2008 trial season limited the scope of the trial. Firstly, seeds of several selected varieties could not be obtained from their respective sources due to lack of availability. Therefore only six of the ten selected varieties could be included in the trial. Furthermore, the issues with funding distribution described in Chapter 5 had an even greater impact on the advanced trials of 2007-2008 than on the multi-location trials. Priority was given to the multi-location trials since one of their goals was to demonstrate variety performance to farmers, and therefore if limited funds were available for pre-financing, NARS partners were encouraged to skip the advanced trial in favor of the multi-location trial. As a result, trials were not conducted in Burkina Faso, Niger, and Senegal, and in Mali AVRDC conducted a trial but IER did not. Finally, the trial in Ghana suffered from the accidental closure of an irrigation lateral and completely dried up prior to the first scoring. As a result, only three advanced trials were conducted in 2007-2008. Nonetheless, the data from these trials do help to further evaluate the performance of the TYLCD-resistant cultivars selected in the previous year's preliminary trial, and help to differentiate between their performance and characteristics.

Materials and Methods

Plant Materials

Of the 28 cultivars evaluated in the 2006-2007 preliminary trial, ten were selected for inclusion in the advanced trial based primarily on their demonstrated resistance to TYLCD. Unfortunately, four of those varieties were not available and therefore could not be included in the trial. They are Aegean and Hamoud Mumyes from Enza Zaden, and DRW 7215 F1 and Industry DR 10401 from De Ruiter Seeds. The remaining six varieties included in the trials are shown in Table 7.1. Roma VF was used again as the susceptible check.

Trial Locations

As mentioned above, advanced trials were conducted in only three locations in 2007-2008 (Figure 7.1). Two of these locations, in Kargui, Benin and Dapaong, Togo, were the same sites used for the concurrent multi-location trials. This came with the advantages of allowing farmers to more easily see the trials, but came with the disadvantages of less intensive management practices that reduced yields and fruit

Table 7.1 – Varieties included in the 2007-2008 advanced trial and their sources. Check marks indicate inclusion in a given trial. Trials were conducted in Benin (BEN), Mali (MAL), and Togo (TOG).

Seed Source	Variety	BEN	MAL	TOG
De Ruiter Seeds	Athyla F1	✓		✓
	Dennolino F1	✓	✓	✓
	Porfyra F1	✓	✓	✓
Enza Zaden	Espadilha	✓	✓	✓
	Sensei	✓	✓	✓
	Setcopa	✓	✓	✓
Tropicasem	Roma VF	✓	✓	✓

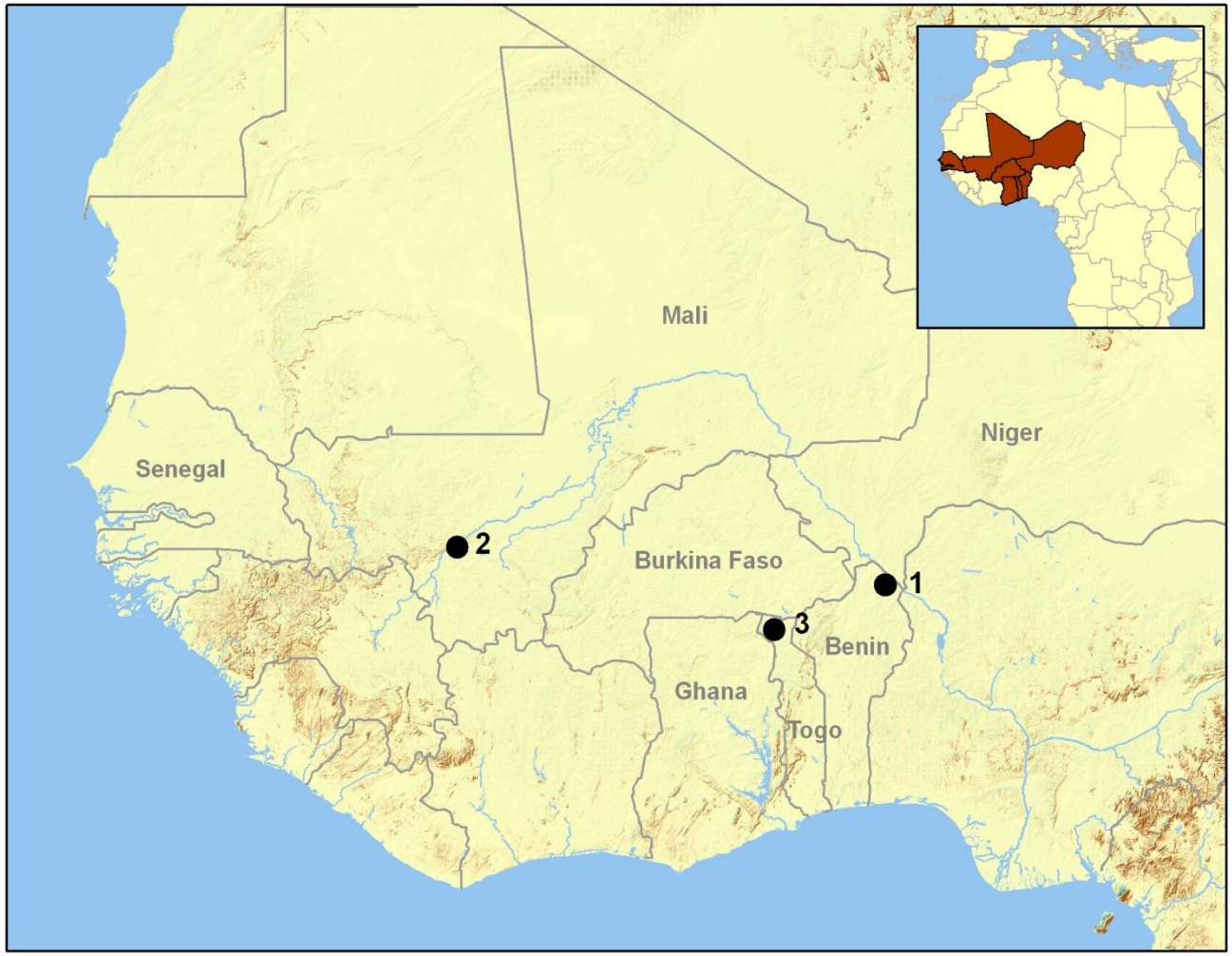

Figure 7.1 – Map of the 2007-2008 advanced trial locations. 1 – Kargui, Benin; 2 – Samanko, Mali; 3 – Dapaong, Togo.

qualities and increased the development of TYLCD symptoms on infected plants, as shown in Chapter 5. The trial in Mali was conducted at the AVRDC research station in Samanko.

Trial Establishment and Management

The trial protocol was very similar to that of the concurrent multi-location trial, with specific instructions related to seedling nursery establishment, site selection, and field management (see Chapter 5 for details). As in the multi-location trials, elementary plots consisted of rows of 12 plants of the variety under evaluation surrounded by spreader rows of Roma VF to ensure uniform disease pressure. A total of five replications were performed, with each block containing all six test cultivars plus a plot of Roma VF.

Disease scoring and yield calculations

Disease scoring was again performed at flowering, fruiting, and first harvest, according to the symptom severity scale described in Chapter 3 (Figure 3.2). Total and commercial yields were also collected as per the 2006-2007 trial protocols. Collection of yield characteristics were performed as described for the concurrent multi-location trials. Three plants in each elementary plot in the selected location were tagged prior to fruiting, so as to avoid biasing data towards particularly high-yielding plants. The number of fruits per plant and the number of fruits per cluster were calculated for each marked plant. In addition, 15 fruits were randomly selected from each variety's yield, and those fruits were measured for weight in grams, and length and diameter in millimeters.

Statistical analysis of yield data was again performed using R. ANOVA and Tukey multiple comparison testing were used to identify varieties that differed significantly by yield characteristics. Box-Cox transformation was used, when necessary, to normalize data prior to analysis.

Results

Benin (INRAB)

The Beninoise advanced trial was conducted in Kargui, in the far north of the country near the city of Malanville and the borders with Niger and Burkina Faso. Seeds for the trial were planted on Dec. 4 2007, and seedlings were transplanted on Jan. 11 2008.

TYLCD pressure on the advanced trial was moderate (Figure 7.2), as seen additionally on the concurrent multi-location trial. In both trials TYLCD pressure built steadily over the course of the season, and by first harvest Roma VF had developed a symptom

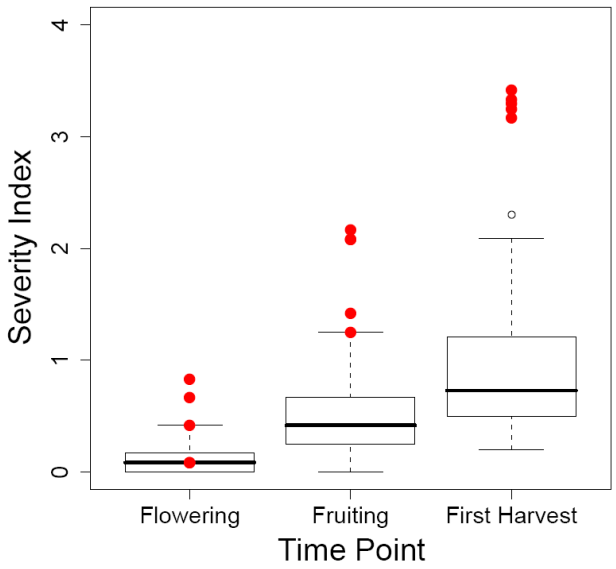

Figure 7.2 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Kargui, Benin. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

severity score of 3.3. All varieties in the trial had symptom severity scores of less than 1.0 at that time point with the exception of Espadilha, which had a score of 1.2. Thus all varieties in the trial demonstrated significant resistance as compared with Roma VF.

Yields were reported in Benin, and ranged from 16.4 t/ha for Setcopa to 9.1 t/ha for Porfyra F1. Varieties were not found to differ significantly by yield. However, significant differences were found between some of the marketable yields: Setcopa and Sensei were found to have significantly higher marketable yields than Espadilha and Porfyra F1. The Beninoise trial partners did report disease and pest incidences on different varieties, and while Espadilha and Porfyra F1 had slightly higher levels of *Helicoverpa* damage, this isn't enough to explain the observed differences. This might imply, though, that Setcopa and Sensei have better keeping qualities or resistance to other pests and diseases than Espadilha and Porfyra F1.

Mali (AVRDC)

The AVRDC advanced trial was conducted at the Samanko research station near Bamako, Mali. Since it was on a research station the trial showed lower disease pressure and higher yields than the trials conducted on rented farmland in Benin and Togo. The AVRDC multi-location trial was conducted off the research station, and therefore results cannot be compared.

By first harvest Roma VF developed an average TYLCD symptom severity score of 3.3, but no other cultivars in the trial showed any disease symptoms (Figure 7.3). Yields were calculated, and ranged from 29.5 t/ha for Dennolino F1 to 15.0 t/ha for Roma VF. Dennolino F1, Sensei, and Setcopa, which all had yields in the 29 t/ha

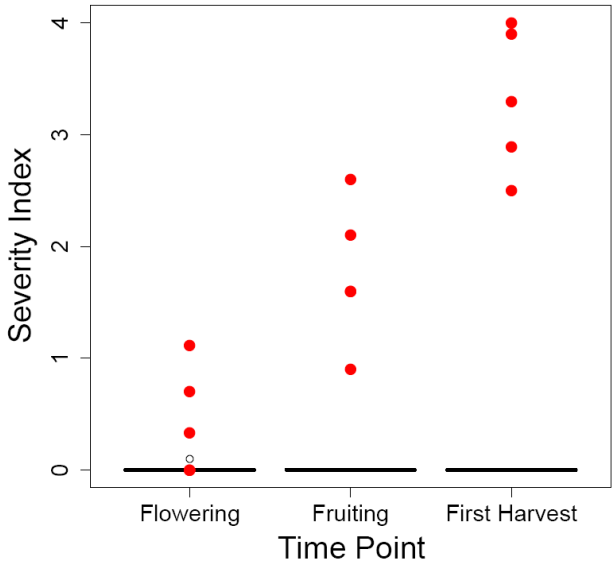

Figure 7.3 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Samanko, Mali. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

range, were found to differ significantly in yield from Roma VF. Marketable yields ranged from 25.0 t/ha for Dennolino F1 to 6.7 t/ha for Roma VF. Dennolino F1, Sensei and Setcopa were again found to differ significantly from Roma VF. In addition, Dennolino was found to have a significantly higher marketable yield than Porfyra F1.

Togo (ITRA)

The Togalese advanced trial was conducted side-by-side with the multi-location trial in Dapaong, in the far north of the country near the border with Burkina Faso. Seeds for the trial were sown on Dec. 7, 2007, and seedlings were transplanted on Jan. 4, 2008. Symptom severity scorings were conducted on Feb. 18, March 13, and Apr. 2, 2008.

As with the concurrent multi-location trial, the Togalese advanced trial showed moderate disease pressure, but relatively uniform responses across all cultivars (Figure 7.4). At first harvest, Roma VF received a TYLCD symptom severity score of 2.8, implying low disease pressure. However, all cultivars under evaluation received scores over 1.5, higher than the scores they received under much higher disease pressure in Benin and Mali. Therefore, it seems that once again the TYLCD scorings in Togo did not necessarily accurately reflect susceptibility to TYLCD, but instead reflected the presence of some other disease which confounded symptom severity measurements.

Yields were reported for the trial and were exceedingly low, ranging from a high of just 4.2 t/ha for Setcopa to a low of 1.0 t/ha for Porfyra F1. Marketable yields were nearly the same as total yields. For both measures, Setcopa was found to have a significantly higher yield than Porfyra F1.

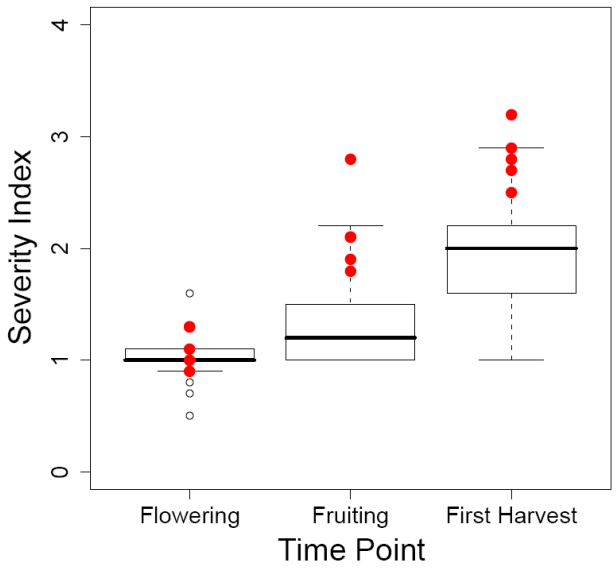

Figure 7.4 – Box and whisker plot of the distribution of TYLCD symptom severity measures at three time points in Dapaong, Togo. Red dots represent the TYLCD symptom severity scores of each plot of Roma VF.

Discussion

Despite only being conducted in three locations throughout West Africa, the 2007-2008 advanced trial did demonstrate that the selected tomato cultivars could perform significantly better than Roma VF both on agricultural research stations and in farmers' fields. In all locations all tested cultivars had lower symptom severity scores than Roma VF, and in Benin and Mali, where disease scores were likely to represent response to TYLCD, tested cultivars had very low symptom severities or even remained symptomless (Table 7.2).

Table 7.2 – TYLCD symptom severity scores of all tested cultivars at first harvest in Benin (BEN), Mali (MAL), and Togo (TOG). Colors are assigned separately for each trial on a linear scale with the lowest value being colored yellow and the highest value being colored blue.

Variety	BEN	MAL	TOG
Athyla F1	0.6		1.6
Dennolino F1	0.6	0.0	1.7
Espadilha	1.2	0.0	1.8
Porfyra F1	0.9	0.0	2.2
Roma VF	3.3	3.3	2.8
Sensei	0.6	0.0	1.7
Setcopa	0.7	0.0	1.8

Table 7.3 shows various yield characteristics for the cultivars evaluated in the trial, including total yield, fruit weight, length, and diameter, number of fruits per plant, and the length / diameter ratio, which gives an approximate measure of fruit shape. While no significant differences were found between yields, it is notable that Sensei, Setcopa and Dennolino F1 had higher yields than other cultivars in all trials. While Roma VF did have the highest number of fruits per plant, its fruits were also the lightest and the

Table 7.3 – Yield and fruit characteristics for all varieties in the 2007-2008 advanced trial. Total yield is measured in t/ha; weight is measured in g; and length and diameter are measured in mm. FPP = fruits per plant, NS = no significant differences found. Superscript letters represent groupings supported by a Tukey HSD test at $p < .05$.

Variety	Total Yield[NS]
Setcopa	22.91
Sensei	22.73
Dennolino	22.51
Espadilha	17.84
Athyla F1	16.16
Roma VF	16.08
Porfyra F1	15.33

Variety	Weight
Porfyra F1	108.1[a]
Athyla F1	85.4[ab]
Setcopa	82.6[ab]
Dennolino	70.9[bc]
Espadilha	70.3[bc]
Sensei	62.1[bc]
Roma VF	47.4[c]

Variety	Length
Roma VF	65.3[a]
Porfyra F1	52.1[b]
Setcopa	46.0[bc]
Dennolino	45.6[bc]
Athyla F1	45.5[bc]
Espadilha	44.7[bc]
Sensei	40.6[c]

Variety	Diameter
Porfyra F1	57.8[a]
Athyla F1	57.3[a]
Setcopa	56.0[a]
Dennolino	49.9[ab]
Espadilha	53.7[ab]
Sensei	46.9[b]
Roma VF	37.3[c]

Variety	FPP
Roma.VF	35.9[a]
Dennolino	29.9[ab]
Sensei	25.4[bc]
Setcopa	20.2[cd]
Athyla.F1	18.4[de]
Espadilha	18.4[de]
Porfyra.F1	14.0[e]

Variety	L/D Ratio
Athyla F1	0.79
Setcopa	0.82
Espadilha	0.83
Sensei	0.87
Porfyra F1	0.90
Dennolino	0.91
Roma VF	1.75

narrowest. Notably, Roma was the only plum-shaped tomato selected, with all others being spherical to flattened.

Since this advanced trial was conducted during the third and final year of the ABSPII trialing project, no varieties were selected for multi-location trials. Rather, efforts have begun to connect the sources of the best varieties in both rounds of trials with seed distribution channels in West Africa. These efforts are described in detail in the next chapter.

CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS

Introduction

The identification of high-yielding tomato varieties with strong resistance to the tomato-infecting begomoviruses of West Africa creates new opportunities for the revival of the tomato processing industry in West Africa. However, several challenges lie ahead. For the potential impact of these varieties to be realized, seeds need to find their way into local seed distribution channels. The seed sector of West Africa is unfortunately significantly underdeveloped (Rohrbach et al., 2003), and thus efforts will need to be invested in the establishment of a regional seed industry. This industry will need to be widely distributed to ensure access by the poorest farmers who may live farthest from urban areas. Furthermore, the canneries in the region will need to be reopened in a timely manner to ensure that the higher yields produced by farmers who have adopted the new varieties do not end up in the fresh market where they will drive prices down and cause all farmers, but especially the poorer, more risk-averse non-adopters, to lose money.

These challenges, while significant, can be met thanks to several programs that are currently in motion in West Africa. This section describes the current and future work being invested in the development of seed dissemination channels in West Africa and the revival of the tomato processing industry. The potential economic impacts of this work are also discussed.

Seed Distribution and the West African Seed Alliance

A variety of historical circumstances have left seed distribution networks in West Africa severely underdeveloped (Rohrbach et al., 2003). During colonial and post-colonial times, seed increase and distribution tended to be controlled by a single government-run entity in each separate country in the region, with little competition allowed. Liberalization of seed markets as a result of structural adjustment programs in the early 1990s opened up opportunities for development of commercial seed enterprises (Gisselquist and van der Meer, 2000), but several factors have discouraged entry into the market. Firstly, regulation of the seed industry has differed from one country to the next, often inhibiting the movement of seed across borders and making it difficult for distributors to attain the economies of scale necessary to enter into such a low-margin business (Rohrbach et al., 2003). International aid efforts have been an additional barrier: while donated seed offers an immediate solution to scarcity, it destroys incentives for commercial seed distribution by offering consumers a free alternative to purchased seed (Tripp and Rohrbach, 2001). Furthermore, the efforts of many NGOs have served as an impediment by focusing on the development of local-scale seed increase and distribution networks; while these networks are highly valuable for the preservation of landraces, they discourage competition and limit seed availability to a narrow range of local cultivars (Tripp and Rohrbach, 2001). Finally, a general lack of access to startup capital for small businesses has made it difficult for small seed enterprises to finance their operations at or beyond a minimum efficient scale of operation, while a lack of available credit for farmers has slowed the development of a market for improved varieties.

Recent efforts to encourage the development of commercial seed enterprises in West Africa have taken a three-pronged approach. On the regional scale, efforts have

focused on the harmonization of seed policy and regulations to establish a protocol for the movement of seeds between countries and to reduce the barriers preventing a seed distributor from operating in more than one country in the region. Work has also begun in the development of regional foundation seed production capacity to facilitate the increase of high-quality seed directly in West Africa. Finally, programs have been implemented to provide resources and training to a network of agro-dealers throughout West Africa who provide the link between centralized resource access points and the smallholder farmers distributed throughout the region. These three approaches are being coordinated in part by the newly established West African Seed Alliance (WASA), a USAID-funded project with the goal of establishing a sustainable commercial seed industry in West Africa to ensure that farmers have "affordable, timely and reliable" access to high quality seeds (CNFA, 2009).

Harmonization of Seed Policy

There are several organizations worldwide that coordinate the harmonization of seed policy between nations (Rohrbach et al., 2003). For instance, intellectual property rights for plant varieties are protected by the International Union for the Protection of New Varieties of Plants (UPOV) convention (UPOV, 1997), while the Trade-Related Aspects of International Property Rights (TRIPS) agreement, administered by the World Trade Organization, sets down minimum standards for the regulation of intellectual property of many different forms, including plant materials (WTO, 1994). International conventions also exist for the regulation of phytosanitary control measures: the International Seed Testing Association (ISTA) establishes internationally agreed upon rules for seed sampling and testing and provides an accreditation program for seed testing laboratories (ISTA, 2009), and the International Plant Protection Convention, administered by the Food and Agriculture Organization

(FAO) of the United Nations (UN), is an international treaty setting out regulations to prevent the movement of plant pests and diseases (FAO, 1997).

Historically, the governments of Sub-Saharan African countries have managed all aspects of seed certification independently, and often inefficiently, in some cases requiring all seeds to be tested in field trials for three years by a national research service even if the variety has already been so evaluated in a neighboring country with a similar agroecology. Criteria used by the national varietal release committees to evaluate varieties often additionally lack transparency and may be unevenly applied (Rohrbach et al., 2003). In contrast, many non-African countries use truth-in-labeling laws to enforce seed quality measures. The United States, for example, allows companies to sell uncertified seeds so long as their labels truthfully report relevant information such as germination rates and weed content as determined by the company's own field supervision and laboratory tests, and the European Union has similar regulations for vegetable seeds (Gisselquist and van der Meer, 2000). However, steps have been taken in recent years to move African nations towards the adoption of international standards for plant movement and varietal release. With regards to vegetable seeds, the African Seed Trade Association (AFSTA) released a position paper in 2003 recommending the adoption of truth-in-labeling laws to allow companies to take on the burden of seed quality certification on their own, thereby easing and expediting the process of vegetable seed release in Sub-Saharan Africa (AFSTA, 2003). In May 2008 the members of the Economic Community of West African States (ECOWAS) adopted a regional agreement aimed at facilitating cross-border trade in seeds, which included provisions for harmonized regulation of seed certification and variety release, publication of a regional seed catalogue to list varieties whose seed can be marketed freely in the region, and the establishment of a

West African Seed Committee (COASEM) to facilitate the implementation of the harmonized regulations (FAO, 2008). Many of these regulations are yet to be implemented by participating nations, but WASA has held two workshops in the last year to help provide informational support for policy makers (CNFA, 2009).

Foundation Seed Production

Seeds typically undergo numerous rounds of increase to turn the relatively small quantities of seed generated by breeders into sufficient quantities for commercial sale. It stands to reason that the production of high-quality seeds of high purity depends on very careful controls in the earlier generations of increase to ensure quality and consistency. The term "foundation seed" is typically used to describe the generation or generations that come between breeder seed and commercial seed. While many seed companies perform seed increases in-house or contract them out to qualified companies, public breeding institutions often lack the resources and infrastructure necessary to oversee the process of seed increase. As a result, successful public breeding programs depend on foundation seed programs to produce sufficient quantities of high quality seeds of public varieties for subsequent increase by commercial seed producers (Tripp, 2006). While the tomato varieties selected by the West African vegetable germplasm trialing network in 2005-2008 were primarily commercial hybrids, the recent establishment of a vegetable breeding program at AVRDC in West Africa (described below) emphasizes the need for foundation seed production in the vegetable sector. To address the need for foundation seed in West Africa, WASA helped to establish a foundation seed program in Nigeria in 2008 to produce seed of millet, rice, groundnut, cowpea, and several vegetables (CNFA, 2009). It is expected that availability of these seeds will boost activities of small commercial seed producers in the region in coming years. In addition, the

development of foundation seed production capacity in West Africa raises the possibility of large multinational seed companies turning to African enterprises for their seed increase needs in the future.

Resources for Agro-Dealers

While regional seed policy harmonization and the establishment of foundation seed production capacity help create an environment more conducive to the development of a regional seed industry, that development is unlikely to occur without the establishment of educational programs and other resources for seed dealers. In that vein WASA has instituted an expansive program to offer training to small scale agrodealers and to improve their access to high quality materials (CNFA, 2009). Educational programs include a 6-module business management training course focused not only on seed marketing but also on the marketing of fertilizers, tools, and crop protection products, as well as programs on the proper usage of inputs and on the establishment of demonstration plots to more effectively market new products to risk-averse farmers and to demonstrate improved agricultural practices. In 2008, training programs began in Mali, Ghana and Nigeria, with further expansion into Niger and Burkina Faso expected in coming years. In addition, WASA helps to form connections between agrodealers dispersed throughout its target countries and centralized sources of high-quality modern agricultural inputs, ensuring that access to seeds and other inputs is widespread throughout the region. WASA aims to have a network of 850 agrodealers in each of its target countries by the end of the project in 2012 (Maroya N, personal communication).

Financing for Agro-Dealers and Credit for Farmers

While a favorable policy environment and accessible training programs are likely to help facilitate the establishment of a more robust seed distribution system, these efforts might not yield fruitful results if financing is not available to both farmers and small agribusiness entrepreneurs. Agro-dealers require startup capital to build their businesses, and farmers often require credit to purchase inputs. WASA does not directly address these needs, but numerous programs in West Africa have been instituted in recent years to help provide financing in the agricultural sector, including USAID's African Global Competitiveness Initiative for businesses and a "warrantage" credit system established by ICRISAT for small farmers (Tabo et al., 2007). It is notable that both WASA's network of agro-dealers and any new canneries that are established in the region could serve as further sources of credit for farmers hoping to buy seeds or inputs.

Distribution of TYLCV-Resistant Tomato Cultivars in West Africa

The work performed by WASA creates a very favorable environment for the introduction of the high-performing TYLCD-resistant tomato cultivars identified by the West African germplasm trialing network in 2005 through 2008. Representatives of some of the multinational seed companies providing some of the most successful materials in the trials have been put in touch with representatives of WASA, and work is underway to arrange for the sale of selected TYLCD-resistant cultivars throughout West Africa in the near future. Importantly, given the competitive nature of the tomato breeding industry in the developed world, some of the selected varieties are already no longer in use in the countries they were originally bred for, and thus surplus seed can be made available in West Africa at a reduced price that might increase the odds of adoption by poor smallholder growers.

The dynamics of variety adoption are exceedingly important in determining whether the variety introduction will be pro-poor or merely benefit the most well-off farmers. A seminal work by the agricultural economist William Cochrane in 1958 described a process dubbed the technology treadmill governing the benefits to farmers of technology adoption. Cochrane observed that the earliest adopters of a yield-increasing technology are often the most significant beneficiaries, as their output increases without having a significant impact on the market, and thus prices remain the same and their incomes rise. As more farmers adopt the technology and average yields begin to rise, market supply begins to increase, causing prices to drop. The losers, then, are the farmers who adopt the technology last or never adopt at all, as their yields remain at pre-technology (low) levels after the market has shifted to a new post-technology (low) price. Thus, the dynamics of the adoption of yield-increasing technologies are such that farmers must continually adopt newly available technologies to keep pace with the yields of their neighbors and remain profitable (Cochrane, 1958).

Several factors have been found to influence the tendency of a farmer to adopt a new yield-increasing technology. A high perceived risk of adopting a new technology is often a significant disincentive, especially for poor farmers who cannot afford short-term losses in exchange for long-term gains. Plenty of agricultural technologies only offer a yield advantage if used properly, and the possibility of failure is enough to discourage poor farmers from making the investment. Technology adoption can additionally be constrained by remoteness. In areas with poor transportation infrastructure, new technologies can take time to diffuse to less accessible areas, putting those areas in a disadvantageous position on the technology treadmill and

often making them less likely to be well-off (Feder and Umali, 1993; Sunding and Zilberman, 2001).

Several features of WASA's agrodealer development program are pro-poor measures that may help to remove some of the technology adoption disadvantages typically experienced by the poor. Firstly, the focus on demonstration plots and farmer field days could help to dramatically reduce the perceived risk to farmers of adopting the new varieties. It has been shown that farmers effectively learn to extract yield potential from new varieties not only by growing the varieties themselves, but also by observing them being grown by others (Foster and Rosenzweig, 1995). As a result, WASA's program to train agrodealers in the establishment of trial plots may result in the dissemination of information crucial to the most effective use of the new varieties. This could in turn lead to decreased risk assessments of the new varieties by poorer, more risk-averse farmers, thereby increasing adoption rates. In addition, the distributed nature of WASA's agrodealer network could minimize the lower access typically experienced by more remote farmers, thereby allowing the new varieties to more efficiently diffuse to less-accessible areas.

Perceived risks associated with adoption of the new high-yielding TYLCD-resistant tomato cultivars might also be mitigated by programs implemented by the newly reopened tomato canneries such as short-term subsidy programs for the purchase of seeds or contracts guaranteeing a set purchase price for tomatoes. However, for these programs to be successfully implemented the canneries will need to reopen in a timely manner. If the reopening of the canneries is dependent on private sector investment, it will be necessary to demonstrate that the yield potential offered by the new tomato varieties is sufficient to make the canneries profitable again, even in the face of

competitively priced imports from Europe and China. The following section addresses the economics of operating a tomato processing plant in West Africa and the question of profitability in a competitive market.

Reestablishment of the Tomato Processing Industry

A breakdown of the costs associated with tomato paste production both in Europe and in Mali, as reported in a recent analysis published by USAID, reveals the ways in which the operation of tomato canneries might once again become profitable in West Africa. (All values and calculations in this section are from Easterling, 2005 unless otherwise noted.) Per kilogram, the cost of imported European tomato paste in Mali is US$2.25. One kg of tomato paste is made from 5 kg of fresh tomatoes, which in Europe cost the processor only 5¢ per kg due to government subsidies paid to growers (Sumner et al., 2001). Thus one kg of paste costs 25¢ in fresh tomatoes. Of the remaining $2.00 in costs, $1.00 goes to manufacturing costs and margin, 25¢ goes to transportation to Mali ($4,000 per 20 ton container) plus handling in Mali, and 75¢ goes to the 60% import duty currently levied on all processed food items coming into the West African Economic and Monetary Union (WAEMU). Thus, for tomato processing in Mali to again be competitive, its product will need to cost less than $2.25 per kg of paste.

The cost structure of paste production for domestic consumption in Mali is somewhat different than that of paste production for export in Europe. Obviously transportation costs are much lower and import tariffs are not levied on domestic production. However, manufacturing costs in Mali are higher due to the need to import cans and labels and due to the higher cost of electricity compared with that in Europe. It is difficult to accurately estimate the cost of tomato paste production in Mali without a

more detailed study of energy and materials costs. However, Easterling roughly estimates a manufacturing cost of approximately $1.50 per kg of paste produced. For the purposes of our present analysis we will proceed with this estimate, but we will revisit it towards the end of the discussion. If on top of this $1.50 in manufacturing costs we assume taxes and profit margin totaling 25¢, we leave tomato processors in Mali 50¢ to spend on fresh tomatoes for every kg of paste produced, which amounts to 10¢ per kg of fresh tomatoes.

The 10¢ per kg to be spent on tomatoes in Mali are not directly comparable to the 5¢ spent per kg of tomatoes in Europe. While European growers are subsidized, the subsidy amounts to only about 2¢ per kg of tomatoes – in the United States, processing tomatoes are typically valued at about 7¢ per kg. However, in the United States and in Europe, the fresh market and the processing market are entirely independent from one another. In the United States, fresh market tomatoes are typically sold by the farmer for 55-75¢ per kg. This significant price difference is due to differences in the economies of scale between the two tomato types – fresh market tomatoes tend to be indeterminate and therefore highly labor intensive, requiring staking, tying, and staggered harvest, while processing tomatoes, which are typically determinate, need no individual per-plant attention during the growing season and can be mechanically harvested. In West Africa, in contrast, there is currently no distinction drawn between processing tomatoes and fresh market tomatoes. Tomatoes are rarely eaten raw in West Africa, and tend to be used as the base for stews and sauces, which means processing-type tomatoes with their high soluble solids are actually preferred by fresh market consumers. These tomatoes are also preferred by producers for their harder texture, which protects them from damage during transport. As a result, the 10¢

available to a tomato processor to pay for 1 kg of tomatoes in Mali needs to be competitive with the price of tomatoes on the fresh market.

The most basic criterion for establishing the feasibility of reopening the tomato cannery in Mali, according to Easterling, would be the possibility of obtaining a price from farmers of 10¢ per kg of tomatoes. While it is difficult to estimate tomato prices in West Africa due to constant fluctuations, both seasonally and from year to year, Easterling cites a Malian government estimate of 750 FCFA, or about 15¢, per kg. (This value seems plausible based on anecdotal experience.) Thus, if production costs per hectare were to remain the same for farmers, the drop in price by 33% from 15¢ per kg to 10¢ per kg would need to be matched by an increase in yield of 33% to keep the farmers' net revenues constant. Of course the adoption of yield increasing seeds comes with increased costs, and thus yields would need to increase even further. The same Malian report cited by Easterling estimates an average cost per hectare for tomato production in Mali of approximately $770. Assuming yields of 6.9 t/ha, which is the average yield of Roma VF observed during the 2007-2008 multilocation trials throughout West Africa and is additionally a value that conforms with those observed prior to the institution of the host free period in Baguineda (Noussourou et al., 2008), in the absence of a change in production costs tomato yields would need to increase to 10.4 t/ha to keep farmers' net revenue constant. If production costs were to double, yields would need to increase to 18.1 t/ha. These yield increases seem entirely attainable – the average yields of over 16 t/ha recorded for cultivars Atak and Yosra during the multi-location trials reflected typical low-input management practices in which the only increase in production cost would be the cost of the seeds. During the advanced trials of 2006-2007, which were conducted on research stations with reasonably high input, yields for those two cultivars averaged approximately 34 t/ha,

representing an almost 5-fold increase in yield over those demonstrated by Roma VF in low-input conditions. Thus an initial rudimentary analysis implies that, with the introduction of the selected varieties from the TYLCD-resistance trials, tomato yield increases in West Africa will be sufficiently high to maintain or increase farmers' earnings at a price that allows canneries to effectively compete against low-cost imports.

The above analysis does not account for the link between the processing and fresh tomato markets in West Africa. To account for that link, tomato yields would need to be sufficient to keep farmers' net revenues constant while also driving prices in the fresh market down to 10¢ per kilogram to prevent farmers from selling to the fresh market instead of to the factory. Anecdotal evidence shows that the price elasticity of demand for fresh tomatoes in West Africa is relatively low: very significant price fluctuations are observed during the course of the tomato season as supply increases and decreases. As a result, as long as high yields are maintained it is expected that the fresh market would probably not have a significant impact on the willingness of farmers to sell to the cannery – if market prices were to briefly rise above 10¢ per hectare, the flood of excess tomatoes from producers trying to take advantage of the higher price would quickly drive the price back down again.

Easterling's initial assumption of a $1.50 manufacturing cost for one kg of tomato paste in Mali was based on a very rough estimation that paste manufacturing costs are 50% higher in West Africa than in Europe. The ramifications of a change in this value could potentially be significant. A manufacturing cost of $1.40 would allow the market price for tomatoes to be 12¢ per kg, meaning that even with doubled production costs tomato yields would still only need to reach 15 t/ha to allow

processors to compete as buyers in the fresh market. In contrast, a manufacturing cost of $1.60 would drop the target price for tomatoes to 8¢ and would raise the necessary yields to 22.7 t/ha. By the time manufacturing costs were to reach $1.80 per kg, tomato yields would need to reach 45 t/ha to maintain the target price of 4¢ per kg. It is clear, then, that relatively small changes in manufacturing costs could have a significant impact on the viability of the tomato processing industry in West Africa.

While the specific cost is not known, there are several steps that could be taken in tandem with the opening of a processing plant that might help reduce processing costs. The manufacture of tomato paste is primarily an energy-intensive exercise in dehydration, with excess water being removed from tomatoes to yield concentrated solids, or paste. One approach to improving the efficiency of the dehydration process would be to use drier starting materials, i.e. tomatoes with lower water content. The tomato varieties included in the West African TYLCD-resistance trials had a wide range of water contents, and it would be prudent to select those with the lowest water contents for processing, or even to try to introduce some dedicated processing varieties. An additional approach to saving costs in paste-manufacture would be to use more efficient and accessible energy sources to drive the dehydration process. Processing plants are most effectively situated near tomato growing regions, but these rural regions are often without a reliable source of power, sometimes necessitating the use of expensive fuel-based generators. The use of sustainable energy sources in rural West Africa has gained some traction in recent years, especially as the manufacturing costs of power-generating equipment have dropped. The tomato harvest in Sahelian West Africa takes place primarily during the cool dry season, when sunlight is abundant. It stands to reason, then, that the use of solar energy to generate electricity or heat for the tomato paste production process could be an effective method for

decreasing the cost of paste production, thereby increasing the target price for tomatoes and lowering the necessary yield increases.

Until now we have considered whether tomato yields could be sufficient to support a price that could fit within a tomato processing budget, but it is also relevant to question whether total tomato production in Mali would be sufficient for the needs of a cannery. The cannery in Baguineda closed in the mid-1990s not specifically because of low and inconsistent yields, but because those low and inconsistent yields resulted in an insufficient total supply of raw materials to keep the cannery operating profitably. While many of a cannery's costs are at least semi-variable with production level, including the costs of tomatoes, cans, electricity, water, and labor, the cost of installing new processing and canning equipment or of rehabilitating the old equipment will likely be fixed and substantial, and therefore to attract investors there must be strong evidence of profitability. Official statistics in Mali put countrywide tomato production at approximately 75,000 tons in 2006 (FAOSTAT, 2009). Easterling estimates that the total quantity of tomato paste imported yearly to Mali is 28,000 tons, corresponding to 140,000 tons of fresh tomatoes. Thus, if tomato yields were to double, sufficient quantities would be available to meet half of the country's tomato paste demand without significantly changing the dynamics of the fresh market. Assuming 25¢ of net revenue per kg as detailed above, this puts the cannery's potential net revenue at $3.5 million per year. While this is likely an attractive-sounding number for investors, it is unlikely of course that production will reach this level in one year: adoption of new varieties will not be an immediate process and will likely not immediately demonstrate full yield potentials, and issues such as the staggering of the harvest to meet the cannery's scheduling needs and transportation logistics will invariably take several years to work out. However, given the proper

investment, it does seem likely that the operation of a tomato cannery in Mali (and, by extension, any other country in West Africa) could be profitable. If this were determined to be beneficial for the national economy (as discussed below) it might be appropriate for the government to share in the investment in the short term to help attract investors and thus ensure a smooth transition to higher-yielding varieties without major price fluctuations, with the intention of divesting once the cannery became profitable.

Interestingly, the necessity of maintaining the cannery as a significant buyer of tomatoes to prevent a crash of market prices requires that farmers grow varieties with high yield stability. Were all farmers to opt for yield potential at the expense of yield stability, a single shock causing low yields could put the cannery out of business and leave farmers without a sufficient market in subsequent seasons. However, this runs contrary to farmers' typical inclinations – if given the choice between a variety with high yield potential and another variety with high yield stability, it has been shown that poor farmers will tend to choose the higher yield potential (Lybbert, 2006). This may be due to the fact that yield stability only proves to be advantageous in years of high disease pressure, drought, or other short-term shocks, leading the added cost of seeds with high yield stability to appear to be an investment that is unlikely to pay off, and thus giving those seeds a greater perceived risk for poor farmers. Of course the varieties selected in the 2005-2008 variety trials offer significantly higher yield potentials than the popular cultivars in the region, providing farmers with plenty of incentive to adopt the new varieties. However, if higher-yielding, non-TYLCD-resistant materials were to become available in West Africa, it might be necessary for canneries to implement programs to encourage farmers to grow the more yield-stable varieties. This might be as simple as specifying varieties to be grown in a contract

with farmers, a practice that is common in the tomato processing industry elsewhere in the world.

While an analysis of the potential profitability of tomato processing in West Africa given the introduction of new high-yielding TYLCD-resistant tomato varieties shows very promising returns, it does not guarantee investment. However, there is reason to believe that there is significant foreign interest in the tomato processing industry of West Africa. For instance, it has been reported that a Swiss company is interested in reopening the tomato processing facility in Baguineda with a throughput of 100,000 tons of fresh tomatoes (20,000 tons of paste) per year (Nathan-MSI, 2002). Reports have also circulated of an Indian company expressing interest in opening a cannery in Burkina Faso. In Ghana, this type of investment has already taken place – the Pwalugu tomato cannery reopened in February 2007 as a partnership between the Ghanaian government and an Italian tomato processing company. Named the North Star Tomato Company (NSTC), the factory was to buy tomatoes from the northern region of Ghana to manufacture tomato paste for domestic consumption. Unfortunately, due to the as-of-yet unsolved problem of low and inconsistent yields, the factory experienced a variety of market issues: in general tomato production was insufficient to keep the cannery supplied with raw materials, and fresh market traders offered better prices leading farmers to sell their produce for fresh market consumption. In 2008 farmers scaled up their tomato plantings to capitalize on the increased demand created by the cannery, but despite the resultant drop in fresh market prices the cannery could not afford to compete with the fresh market, and did not operate. The resultant loss in expected revenue was devastating for farmers, and three tomato farmers in northern Ghana committed suicide in 2008. This clearly illustrates the very important need to coordinate the establishment of tomato processing capacity with the introduction of

high-yielding tomato varieties in West Africa. WASA may be able to help with this coordination in the coming years as it has a goal of using its agrodealer network to link farmers not only with input channels but with output channels as well.

Potential Economic Impacts of the Reestablishment of Tomato Processing Capacity

While it can be demonstrated that the introduction of high-yielding TYLCD-resistant tomato varieties in West Africa would likely allow tomato processing in the region to again be profitable, it is not unreasonable to ask whether this is actually in the best interest of the farmers or the rural or national economies involved. This section explores the potential economic impacts of introducing high-yielding tomato varieties and opening tomato canneries in the region.

In general, the literature strongly supports the notion that growth in the agricultural sector leads to overall economic growth in developing countries, and numerous empirical studies have demonstrated this (reviewed by Irz et al., 2001; and Thirtle et al., 2003). For instance, Ravallion and Datt (1996) examined the poverty alleviation effects of economic growth in different sectors and different areas in India and determined that rural growth reduced poverty in both rural and urban areas, while urban growth had some benefits for the urban poor but had no impact on rural poverty. In addition, they found that agricultural growth had a positive benefit for the poor in both urban and rural areas. Thorbecke and Jung (1996) came to similar conclusions, finding that in Indonesia the agricultural and service sectors contributed much more to poverty alleviation than the industrial sector. Several studies attempt to quantify the effect of rural or agricultural growth on incomes by calculating a multiplier for the effect. For instance, Timmer (1995) demonstrates that in Kenya, an increase in agricultural output raises national incomes by 1.64 times that increase, while an

increase in industrial output has a multiplier of just 1.23. Gallup et al. (1997) conduct a meta-analysis of the relationship between growth and poverty across multiple countries and find that a 1% increase in agricultural GDP leads to a 1.61% increase in the incomes of the poorest quintile, while the effects for similar increases in GDP from manufacturing or services are just 1.16% and 0.79%, respectively.

It is worthwhile to note several characteristics of the planned agricultural growth in West Africa's tomato processing sector that might complicate the accumulation of downstream benefits. Firstly, this growth in agricultural production is occurring in a sector in which the market is already sometimes saturated in some locations. Without the coordinated increase in processing capacity this growth could be expected to have very significant negative short-term impacts as many farmers were forced out of tomato production due to complete saturation of the market, even if long-term benefits were positive. Furthermore, the reestablishment of tomato processing capacity will take place in an already-competitive market which will constrain the potential increase in earnings deriving from increased tomato production. As mentioned above, there is a very significant difference between the impacts of agricultural growth and the impacts of industrial growth, and depending on how the tomato processing sector is viewed it can fall into either of these categories. Despite these potential constraints, it seems likely that if implemented properly the increase in tomato production and processing will have a significant positive downstream impact. The following sections explore the respective impacts on the farm economy, the rural economy, and the national economy.

Farm Economy

The two most elementary consequences of agricultural growth are higher incomes for farmers and higher demand for on-farm labor (Irz et al., 2001). In many cases, higher income for farmers deriving from yield-increasing technologies can disproportionately favor wealthier farmers due to access constraints and higher levels of risk aversion among poor farmers (Hazell and Haddad, 2001). As described above, several elements of WASA's program for agrodealer development specifically address these issues in a pro-poor manner. In the case of tomatoes in West Africa, increased incomes are additionally constrained by the competitive tomato paste market which sets a cap on the price of tomatoes. For the project to be marginally successful, yields will have to rise to at least the minimal level where the price of tomatoes on the fresh market is 10¢ per kg, and where total farm output at that price gives farmers the net revenue they currently earn from tomato production. For yield increases beyond that minimal level, the cannery will be able to absorb excess production and continue to pay farmers 10¢ per kg, thereby avoiding a drop in prices deriving from excess production. This implies the existence of a "sweet spot" in the tomato production distribution at which the price will remain 10¢ per kg on the fresh market and the cannery will be operating at full capacity, generating maximal net revenues for farmers as an aggregate group. This sweet spot will need to be determined empirically, and careful management of harvest schedules and processing capacity will be necessary for its proper execution. However, at this level of tomato production it is expected that farmer incomes will rise significantly. The level of increase in incomes will depend on how much agricultural intensification is necessary to achieve the desired yields: increased need for inputs will reduce net earnings, as will increased need for on-farm labor.

The extent to which higher-yielding TYLCD-resistant tomato varieties will require an increase in on-farm labor is questionable. Higher yields are likely to require more hands for harvesting, and increased input usage will additionally require more labor. However, one report from Mali indicates that tomato farmers currently only devote an average of 20 days per season to their tomato fields (Nathan-MSI, 2002). Thus it is likely that increased labor needs will go primarily to farmers and their family members. However, this may remove farmers and their family members from the local labor pool, reducing the market supply of labor and thus increasing wages.

Rural Economy

The effects of increases in agricultural productivity on rural and national economies are often described as farm-nonfarm linkages, and fall into three categories: upstream production linkage, downstream production linkages, and consumption linkages (Irz et al., 2001).

Upstream production linkages are increased expenditures in the agricultural inputs and services sector as a result of increased farm revenue. These linkages are likely to be highly developed for tomato production in West Africa, thanks in a large part to WASA and its network of agrodealers. Expenditures on seeds will certainly increase, and it is likely that expenditure on inputs such as fertilizers and crop protection products will increase as well. This leads to jobs and increased incomes for agrodealers and product support specialists as well as all those involved in the input supply chain. While these linkages are often affected by the level of infrastructure development in the region, the activities of WASA will likely minimize some of the negative effects of remoteness.

Downstream production linkages are increases in jobs and incomes in agricultural and food processing and related industries. This linkage will certainly be a tremendous driver of growth associated with the introduction of higher-yielding tomato varieties. Canneries nominally need workers to run the canning machinery, but they additionally create jobs in transport of raw materials and finished products, the manufacture of raw materials such as labels and cans, and marketing, to name just a few. Both the upstream and downstream production linkages create higher demand for labor through the creation of jobs, thereby leading to overall increases in wages in the local rural economy.

Consumption linkages, which have been estimated to represent as much as 75% of all farm-nonfarm linkages, are increased jobs and incomes that arise as farmers and laborers spend their increased incomes on goods and services in the local rural economy. Calculated multiplier effects for these linkages in Sub-Saharan African countries have ranged from 1.3 to as much as 4.6 (Delgado et al., 1994; Delgado et al., 1998; Haggblade et al., 1991). Given the number of different sectors expected to benefit from the increase in tomato production and the establishment of processing capacity, it is expected that consumption linkages will be very significant.

There are several other linkages that may be relevant to the growth of the rural economy following development of the tomato industry in West Africa. For instance, increased incomes may lead to increased expenditures on food, health and education that will, in turn, improve social welfare (Timmer, 1995). Since tomato is not a staple crop, the macronutritional benefits sometimes seen from lower food prices will likely not apply, but lower prices of tomatoes could well improve micronutrient intake in rural areas by increasing vegetable consumption. The required scheduling of the

tomato harvest over a longer period of months to ensure optimal efficiency of the canning operation will additionally lead to the availability of inexpensive vegetables over a longer period of time each year, further improving micronutrient intake. A shift from reliance on imported tomato paste to domestic production will cause a shift in the source of tax revenues from import duties to locally collected taxes from the cannery, farmers and laborers with higher incomes, and/or sales. While the magnitude of the difference in total tax revenue will depend in a large part on each country's tax regime, the shift to more locally collected taxes may be of significant rural benefit in areas with strong regional and local governments. The needs of the processing industry will create demand for improved infrastructure such as roads and energy supply, and strong local governments will likely capitalize on the increased tax revenues to initiate infrastructure development projects. Finally, the improved dynamics of the farm sector deriving from increased interactions between agrodealers and input consultants, farmers, processors, and banks may lead to the formation of social capital as these parties gain confidence in working together and pursue further, non-agricultural businesses.

National Economy

The impact of agricultural growth on the national economy derives primarily from the accumulation of capital within the agricultural sector and the subsequent transfer of that capital to other sectors both through purchases and through investment (Irz et al., 2001). Investment can take the form of savings by the agricultural population, but governments often accelerate the process by taxing agriculture directly or indirectly. According to Schiff and Valdés (1992), agriculture makes significant contributions to net government revenue in developing countries.

The development of increased tomato processing capacity will additionally offer a domestic product to replace an imported one, thus slowing the export of foreign exchange and increasing opportunities for the import of capital goods that may be critical for further development projects and cannot be manufactured in West Africa (Irz et al., 2001).

Future Activities of the West African Vegetable Germplasm Trialing Network

The establishment of a region-wide coordinated vegetable germplasm trialing network in West Africa has allowed participating countries to evaluate tomato cultivars for true region-wide adaptation in a way that had not previously been possible. This is particularly relevant given recent efforts to harmonize seed policy and regulation across the region. The continued operation of this network offers the promise of more rigorous varietal screening procedures focusing on the traits that are of greatest importance on a regional scale, freeing up national efforts to focus on more local issues and increasing the efficiency of wide-scale varietal introduction in the region.

While the identification of high-yielding TYLCD-resistant tomato cultivars suited for growth throughout West Africa is a significant accomplishment, there are still many opportunities for further improvement of tomato. The trials have allowed the research partners to identify other highly pressing diseases of tomato that are serious constraints to production in West Africa. For instance, fungal diseases are a particularly significant problem in the humid southern areas, and bacterial wilt and root knot nematodes can be found everywhere throughout the region. Trials aimed at identifying materials resistant to those diseases would likely have a significant impact, especially for tomato farmers in the south. In addition, the trialing network now has

the opportunity to begin working on other vegetables of economic importance in the region. Onions, peppers, okra, and cabbage are all of particular importance.

A recent program initiated by AVRDC has begun to develop vegetable breeding capacity in West Africa. Called Vegetable Breeding and Seed Systems (vBSS) and funded by the Bill and Melinda Gates Foundation, this program has begun breeding efforts in several locations in Sub-Saharan Africa, including at the AVRDC research station in Samanko, Mali. Work is under way to develop cultivars of commercially important vegetables as well as indigenous vegetables (AVRDC, 2008). It will likely be some time before materials from this program are ready for distributed trials, but when they are the vegetable germplasm trialing network may offer an ideal resource for coordination of the evaluation of those materials throughout West Africa.

Summary

Since the late 1980s, the tomato production and processing industries of West Africa have suffered from poor yields and unstable markets. While many different factors may have a role in this situation, the lack of modern varieties with resistance to Tomato Yellow Leaf Curl Disease (TYLCD), one of the most debilitating constraints to tomato production in the region, were identified as particularly weak points that could easily be addressed. In 2005-2008 a series of variety trials was conducted in seven countries in West Africa to evaluate over 100 modern tomato cultivars from public and private sources with putative resistance to TYLCD. Managed by a newly formed West African vegetable germplasm trialing network, these trials served as much as an opportunity for training as one for variety evaluation. By 2008 several TYLCD-resistant cultivars with consistently high yields across multiple environments were selected by the trialing network for introduction in the region. Work is now

under way, through a partnership with the West African Seed Alliance, to make those varieties available to farmers and to encourage the reestablishment of tomato processing capacity in the region.

APPENDIX 1

WEST AFRICAN BIOTECHNOLOGY TRAINING WORKSHOP

The Agricultural Biotechnology Support Project II (ABSPII), which was the primary source of funding for the development of the West African vegetable germplasm trialing network and for the execution of the TYLCD-resistance trials, defines its primary mission as the use of biotechnology for the support of agricultural development. While none of the TYLCD-resistant tomato cultivars included in the variety trials were transgenic, several other avenues of biotechnology support were provided to the participating West African countries. The centerpiece of this support program was a week-long intensive biotechnology training workshop, conducted in Bamako, Mali in August 2007, to train NARS research scientists basic molecular biology theory and techniques to help them develop further research capacity in their countries. Twenty one participants from all seven ABSPII partner countries attended the workshop, which was held in the biotechnology laboratory at the University of Bamako. The following pages contain the syllabus from that training workshop.

Note: All lecture topics taught by the author are marked with an asterisk in the detailed syllabus below. Laboratory exercises were collaboratively taught by both the author and Kari Perez.

Molecular Biology for Agricultural Research Applications

August 27th – 31st, 2007 – Bamako, Mali

Introduction

This document outlines a curriculum for an intensive hands-on workshop on molecular biological theory and techniques to be held in Bamako, Mali August 27th – 31st, 2007. The workshop will introduce the ABSPII West African NARES partners, who have been involved in conducting trials of begomovirus-resistant tomatoes, to applications of molecular plant genetics and pathology through integrated lectures and laboratory exercises. The goal of the workshop is to build regional intellectual capacity in West Africa for the continuation of ABSPII-related activities, including modern plant breeding, germplasm screening, and pathogen detection by scientists and practitioners in the region. Structured to acknowledge the conditions of the research programs in the participants' countries while simultaneously building expertise for future capacity expansion, the workshop's laboratory exercises cover techniques with minimal equipment requirements alongside the state-of-the-art approaches to transgene detection and pathogen identification.

Course Details

Instructors

Jeff Gordon, graduate student, Cornell University. jsg54@cornell.edu
Kari Perez, graduate student, Cornell University. kwp6@cornell.edu

Location

Biotechnology Laboratory, University of Bamako, Mali
Dean of the Faculty of Science and Technology: Saliku Sanogo
Director of the Biotechnology Laboratory: Ousmane Koita

Onsite Coordinators

Dr. Issoufou Kollo Abdourhamane, Project Coordinator, AVRDC
Dr. Ousmane Cisse, University of Bamako
Dr. Youssouf Sanogo, University of Bamako

Course Outline:

The course will be conducted over a period of five days, with all days having both lecture and laboratory components. The following is a breakdown of the topics covered by day:

Section Topics:

1. Basic plant molecular biology and genetics
   Associated laboratory exercise: getting acquainted with the lab
2. Basic techniques for detecting, manipulating and identifying nucleic acids
   Associated laboratory exercise: DNA extraction
3. Genetic engineering and plant transformation
   Associated laboratory exercise: DNA detection – PCR and gel electrophoresis
4. Basic molecular plant pathology and disease resistance
   Associated laboratory exercise: DNA detection – Squash blots
5. Detection of proteins
   Associated laboratory exercise: Protein detection – ELISA and immunostrips

Day 1: Basic plant molecular biology and genetics

This section will serve as an introduction to the biological concepts necessary for subsequent sections of the workshop. Of course, it is impossible to teach all of plant molecular biology in one day, and therefore this section will be carefully designed to emphasize the aspects of plant molecular biology that are most relevant to the techniques covered in the workshop.

Topics:

- Introduction to the plant cell
- From DNA to protein – transcription and translation*
- Cell cycle: Mitosis and meiosis
- Molecular genetics: Mendel meets DNA*

Laboratory Exercise: Getting acquainted with the lab

Workshop participants may be unfamiliar with the basic tools, facilities, and safety procedures of molecular biology labs. This laboratory section will be used to ensure that participants are ready to begin conducting experiments by day 2.

- Tour of laboratory equipment and facilities
- Overview of laboratory safety regulations and protocols
- Pipetting practice

Day 2: Basic techniques for detecting, manipulating and identifying nucleic acids

Detection and manipulation of nucleic acids serve as the foundation of molecular genetics. In this section, workshop participants will learn about the basic toolkit available to molecular geneticists from a practical standpoint.

Topics:

- Theory and practice of DNA extraction*
- Hybridization – using complementarity for specific detection*
- Cutting and pasting – restriction enzymes and ligases*
- Separation – gel electrophoresis*
- Amplification – PCR, RT, and bacterial amplification*
- Combining techniques for in-depth analysis – Mapping, cloning, and sequencing*

Laboratory Exercise: DNA Extraction

Laboratory exercises for days 2 will focus on three different methods for extracting DNA from plant materials. Each method meets different scientific needs and is appropriate for different analyses:

- CTAB method – used to purify DNA from fresh leaf tissues in the laboratory. Is appropriate for most downstream applications.
- FTA cards – used for collecting samples in the field, and for long-term storage of DNA or RNA at room temperature. Can be used as templates for DNA or RNA amplification.
- Squash blots on nylon membranes – also used for collecting samples in the field. They are appropriate for detection of specific DNA sequences by hybridization.

Day 3: Genetic engineering and plant transformation

The lectures on day 3 will focus on how and why transgenics are made. Genetic engineering of plants can potentially offer solutions to agricultural problems in the developing world, from virus resistance to drought tolerance. It is also a major tool in modern molecular biology, and a capacity that is relevant to modern laboratory research even when no agricultural product is intended. This section will elucidate the basic processes involved in generating transgenic plants.

Topics:

- Why transgenics – crossability barriers and the linkage problem*
- Genetic engineering – design and assembly of a transgene cassette
- Transformation – Agrobacterium and gene guns*

Laboratory Exercise: DNA Detection

Laboratory exercises for day 3 will introduce two different approaches for detecting specific sequences in DNA samples. For these exercises participants will use the DNA samples they extracted the previous day.

- Transgene detection by PCR: Polymerase chain reaction (PCR) is a powerful technique used for amplifying short, specific sequences of DNA for detection or further manipulation. Participants will use PCR to amplify the sequences for both a transgene and a housekeeping gene from both transgenic and wild-type DNA samples. Agarose gel electrophoresis will be used to visualize the PCR results.

- Geminivirus detection by squash blot: Squash blots allow for the direct detection of high-copy sequences, such as viral genomes, in tissue samples collected in the field. Participants will be use squash blots to search for geminiviruses in plants collected in Bamako. On day 3 participants will set up the squash blot hybridizations.

Day 4: Basic molecular plant pathology and disease resistance

This section will mark a change of focus from the theory and practice of molecular biology to the more applied topic of plant pathology. Pathogens are a serious constraint to production, and appropriate control depends on proper pathogen identification. A special session on day 4, led by Dr. Issoufou Kollo Abdourhamane, will introduce participants to many of the pathogens endemic to West Africa. Lectures will address the different types of pathogens, their impacts on plant function, and plant defense responses, with a focus on the translation of molecular processes into visible symptoms.

Topics:

- Fungi, nematodes, bacteria and viruses – an overview of plant pathogens
- Local plant diseases – an introduction to West African pathogens
- Molecular disease – pathogen effects on cell and molecular processes*
- Resistance genes – dominant vs. recessive resistance

Laboratory Exercise: Squash Blot Detection of Geminiviruses (cont'd)

Workshop participants will continue the squash blot protocol started on day 3 by conducting various washes of the blots, and setting up the color development reaction.

Day 5: Detection of proteins

The detection and analysis of proteins is vital to molecular biology. While the tools of genetics offer scientists many opportunities to study protein function indirectly, it is often necessary to more directly detect, isolate, and manipulate proteins to better understand their functions. The session will begin with a study of antibodies and their use in diagnostic aspects of molecular plant pathology, and will continue to cover more advanced protein analysis techniques and their uses in modern proteomics studies.

Topics:

- Antibodies – what are they?
- Antibody production and purification
- Protein detection methods using antibodies
- Advanced protein analysis*

Laboratory Exercise: *Ralstonia* detection in tomato

Ralstonia solanacearum is a bacterial pathogen that causes bacterial speck disease in tomato. It can be detected using DNA or protein methods – in this session we will be using two different protein methods to detect *Ralstonia* in samples collected from around Bamako.

- Enzyme-Linked ImmunoSorbent Assay (ELISA) is a sensitive laboratory technique for detecting proteins with specific antibodies. Antibodies are linked to an enzyme that generates a colored precipitate when exposed to a colorless buffer, allowing detection of very low levels of the protein being sought.
- Immunostrips are modern protein detection kits designed for use directly in the field. Though based on similar chemistry to ELISA, they sacrifice the flexibility and semi-quantitative nature of ELISA for extremely high speed and ease of use.

APPENDIX 2
TRIAL DATA

Year 1 Preliminary Trial – Cotonou, Benin

Variety	Sev1	Sev2	Sev3
Atak	0.3	1.2	2.2
Bybal	0.2	1.4	2.3
Chenoa	0.3	1.3	3
Cheyenne.E448	0.2	1.6	2.2
CLN.2123A	0	0.9	2.3
CLN.2460E	0	1.8	2.1
CLN.2468A	0	1.6	2.3
CLN.2498E	0	1.5	2.2
CLN.2545A	0.3	1.7	2.4
CLN.2545B	0.1	1.9	2.3
F1.3019.Galina	0	1.7	2.1
Favi.9	1	2	2.4
FTC.6231	0.2	1.3	2.4
FTC.6236	0.1	1.3	2.2
FTC.7088	0.6	1.6	2.8
FTC.7127	0	1.6	2
FTC.7351	0.8	2.3	2.6
FTC.7483	0.3	1.5	2.3
GemPride	0.5	2	2.1
HA.3060	0.4	1.8	2
HMX.4810	0.6	1.7	2.5
Industry.DR.10403	0.4	1.6	2.4
Lety.F1	0.9	1.8	2
Nadira	0	1.8	2.1
Nirouz.TH.99806	0.2	1.8	2
O4.108	0.3	1.9	2.1
O4.240	0.4	2.2	2.1
O4.495	0.2	1.6	2
O4.498	0.7	2.1	2.2
O4.501	0.8	1.9	2.4
Ponchita	0.6	1.8	2.2
PS.43316	0	2	2
PT.4722A	0.2	2.1	2.4
Realeza	0.2	1.1	2.4
Roma.VF	0.1	1.5	2.4
Thoriya	0.5	1	2.1

Variety	Sev1	Sev2	Sev3
TLCV.15	0	1.8	2.2
TY.75	0.3	1.8	2.5
Yassamen.TH.99802	0.3	2	2.5
Yosra	0.2	1	2.3

Year 1 Preliminary Trial – Kou Valley, Burkina Faso

Variety	Sev1	Sev2	Sev3	Total Yield
Atak	0.1	0.7	1.8	6.3
Bybal	0	0.6	1.5	7.4
Chenoa	0	1.3	1.6	3.8
Cheyenne.E448	0	0.5	1.7	8.8
CLN.2123A	0.1	3.2	4	1.5
CLN.2460E	0.1	2.6	3.7	1.3
CLN.2468A	0.4	3.2	3.5	1.1
CLN.2498E	0.2	3.3	3.7	1.4
CLN.2545A	0.1	3.3	3.6	1.9
CLN.2545B	0.2	3.5	3.4	2.8
F1.3019.Galina	0	0.6	2.1	8.1
Favi.9	0	1.5	3.5	3.9
FTC.6236	0	1.3	2.3	7.9
FTC.7088	0	0.7	2.4	4.9
FTC.7127	0	0.4	3.5	1.8
FTC.7351	0	1.9	3.6	1.5
FTC.7483	0	0.4	2.7	2.8
GemPride	0	1.2	2.1	6.6
HA.3060	0	2.3	2	5.3
HMX.4810	0.1	0.8	2.5	7.3
Industry.DR.10403	0	0.3	2.9	7.6
Lety.F1	0	1.7	3	3
Nadira	0	0.6	2.7	5.3
Nirouz.TH.99806	0	0.3	2	9.2
O4.108	0	1.5	2.4	2.1
O4.240	0	1.9	3.2	2.1
O4.495	0	1.6	2.6	3.5
O4.498	0	1.7	3.1	2.2
O4.501	0	1.3	3.3	3.9
Ponchita	0	0.2	1.2	2.9
PS.43316	0	0.5	2.4	5.4
PT.4722A	0.2	1.8	3.7	1.3
Realeza	0	0.4	2.5	4.3
Roma.VF	0.3	3.9	4	0.2
Sasya.0202.F1	0	1.9	3.5	3.7
Thoriya	0	0.6	1.9	7.7

TLCV.15	0	0.2	3.7	1.8
TY.75	0	1.2	1.7	9.1
Yassamen.TH.99802	0	0.5	1.4	10.2
Yosra	0	0.7	2.1	3.7

Year 1 Preliminary Trial – Kumasi, Ghana (Trial 1)

Variety	Sev1	Sev2	Sev3	Total Yield
Atak		2	4	0.7
Bybal		2	3	2.3
Chenoa		2	3	3.2
Cheyenne.E448		2	2	1.9
CLN.2123A		1	2	3.5
CLN.2460E		2	3	2.5
CLN.2468A		2	3	2.6
CLN.2498E		2	3	0.9
CLN.2545A		1	2	3.9
CLN.2545B		1.9	3	2
F1.3019.Galina		2	3	2.3
Favi.9		2	3	9.1
FTC.6231		1	1.8	2.3
FTC.6236		0	1	4.2
FTC.7088		1	2	3
FTC.7127		2	3	1.7
FTC.7351		2	2	6
FTC.7483		3	3	3.1
GemPride		2	2	7.5
HA.3060		1	2	2
HMX.4810		1	3	2
Industry.DR.10403		2	3	2.6
Lety.F1		1	3	1
Nadira		2	3	1.9
Nirouz.TH.99806		1	3	4.1
O4.108		1.8	2	2.2
O4.240		0	1.5	2
O4.495		2	3	1.1
O4.498		1	2	2.2
O4.501		1	3	0.8
Ponchita		2	4	1
PS.43316		1	3	2.6
PT.4722A		1	2	9.1
Realeza		1	1	5.2
Roma.VF		1.6	2.7	1.1
Thoriya		2	2	3.5

TLCV.15	1	3	0.7
TY.75	1	3	2.9
Yassamen.TH.99802	2	2	7
Yosra	0	2	1.6

Year 1 Preliminary Trial – Kumasi, Ghana (Trial 2)

Variety	Sev1	Sev2	Sev3
Bybal		0	3.8
Cheyenne.E448		1	3.4
CLN.2460E		1.7	3.2
CLN.2468A		0	3.3
CLN.2498E		1	3.3
CLN.2545A		1.2	3.6
Favi.9		0	3.3
FTC.6231		0.3	3.3
FTC.6236		0.2	1.6
FTC.7088		0	3
FTC.7127		0.1	3.1
FTC.7351		0.3	1.8
FTC.7483		0.3	3.3
GemPride		0.1	1.5
HA.3060		0.5	3.6
HMX.4810		0.8	3.4
Nadira		0.6	3.4
Nirouz.TH.99806		1.5	3.4
O4.108		0.7	3.1
O4.240		0	3.3
O4.495		1	3.6
O4.498		0	3.1
O4.501		1	3.3
PT.4722A		0.2	3
Realeza		0.5	3.9
Roma.VF		0.7	3.6
Thoriya		1	3.3
TLCV.15		0.8	3.2
TY.75		1.5	3.5
Yassamen.TH.99802		0.8	3.4
Yosra		1.5	1.8

Year 1 Preliminary Trial – Baguineda, Mali

Variety	Sev1	Sev2	Sev3	Total Yield
Atak	0	0	0	5.2
Bybal	0	0	0	3.8
Chenoa	0	0	0	5.2
Cheyenne.E448	0	0	0.8	5.9
CLN.2123A	0	0.3	1.9	5
CLN.2460E	0	0	1.6	4.5
CLN.2545A	0	0.1	1.6	1.3
F1.3019.Galina	0	0	0.6	2.3
Favi.9	0	0.1	2.3	3.4
FTC.6236	0	0.1	1.9	2.6
FTC.7127	0	0.1	1.9	3.8
FTC.7351	0	0.2	2.8	2.3
FTC.7483	0	0.2	0.4	2.3
GemPride	0	0.2	1.4	3.3
HA.3060	0	0.1	0.7	4.1
Industry.DR.10403	0	0	0.7	1.8
Lety.F1	0	0	0.2	1.1
Nadira	0	0	1.1	3.1
Nirouz.TH.99806	0	0	0.8	3.7
O4.108	0	0	2	1.2
O4.240	0	0	0.9	6
O4.495	0	0	1	4.3
O4.498	0	0	1.2	4.1
O4.501	0	0.1	1.6	3.3
Ponchita	0	0	0.1	1.5
PS.43316	0	0	2.1	3.9
PT.4722a	0	0.2	2.9	2.5
Realeza	0	0	0	3.7
Roma.VF	0.1	0.2	2.7	2
Thoriya	0	0.1	0.2	3.7
TLCV.15	0	0	0.2	1.9
TY.75	0	0.2	0.3	2
Yassamen.TH.99802	0	0.1	1.4	1.9
Yosra	0	0	1.5	2.6

Year 1 Preliminary Trial – Samanko, Mali

Variety	Sev1	Sev2	Sev3	Total Yield
Atak	0	0.1	0.3	19.8
Bybal	0	0	0.1	13.1
Chenoa	0	0	0.3	15.2
Cheyenne.E448	0.3	0.5	1.7	17.2
CLN.1466J	1.5	2.8	3.6	4.8
CLN.2123A	0.8	1.3	2.3	13.5
CLN.2460E	0.4	1.5	3.4	13.6
CLN.2468A	0.5	1.4	2.9	7.2
CLN.2498E	0.4	1.3	2.4	8.1
CLN.2545B	0	1.9	3	12.1
CLN.2714-117232961211	1	2.8	3.2	7.7
CLN.2764-82-5-12	0	1.9	3	17.3
CLN.2764-99-13-18	0	0.9	2	18.8
CLN.2768-69-23-30	0.1	0.4	2.2	18
CLN.27777-168-27-2	0.3	1.6	2.4	15.9
F1.3019.Galina	0.2	0.9	1.9	19.1
F1.483	0.3	0.7	2.1	13.8
F1.495	0	0.4	2.1	18
F1.641	0.3	0.1	1.6	18.3
F3.1	0.2	0.7	2.9	16.7
F3.10	0	0	0	14.7
F3.11	0.5	1	2.3	11.8
F3.2	0	0.7	2.8	15.4
F3.3	0.3	0.9	2.2	13.8
F3.4	0	0.1	1.6	9.5
F3.5.	0.4	1.7	3.1	12.1
F3.6	0	0.2	1.8	18.5
F3.7	0.2	1.4	2.9	14.4
F3.8	0.4	0.8	2.1	18
F3.9	0	0	0.2	11.8
Favi.9	0.2	1.3	2.7	17.8
FLA.456-4	0	0	0.5	12.7
FTC.6236	0	0.3	0.5	19.7
FTC.7088	0.3	1	1.8	20.6
FTC.7127	0.4	2.2	3	12.6
FTC.7351	0.4	1.9	3.3	10.1

GemPride	0	0	1.6	19.6
HA.3019	0	0.2	1.7	16.1
HA.3060	0	0.5	2.8	15.8
HMX.4810	0	0.5	2	23.4
Industry.DR.10403	0.2	0.4	1.3	20.3
Lety.F1	0	0.3	0.3	12.5
Nadira	0.4	1.2	2.7	14.6
Nirouz.TH.99806	0	0.1	1.6	23.1
O4.108	0.5	1.2	2.3	14.7
O4.240	0.3	1.5	3.1	20.7
O4.495	0	0.2	2	17.1
O4.498	0.1	0.2	1.8	20.2
O4.501	0.6	2	3.2	13
Ponchita	0	0	0.3	15.8
PS.43316	1	2.5	2.5	14.2
PT.4722A	0.5	2.5	3.3	12.5
Realeza	0.2	0.3	0.5	14.7
Roma.VF	1	2.9	3.5	
Sasya.0202.F1	0.3	1.1	2.8	15.9
Thoriya	0	0	0	18.2
TLCV.15	0.4	0.2	3.2	13.9
TY.75	0	0.2	1.1	15.6
Yassamen.TH.99802	0	0.1	0.3	15.8
Yosra	0	0	0.5	15.7

Year 1 Preliminary Trial – Rufisque, Senegal

Variety	Sev1	Sev2	Sev3	Total Yield
Atak	0	0	0	31.8
Bybal	0	0	0	24.7
Chenoa	0	0	0	28.1
Cheyenne.E448	0	0.5	0.8	44.8
CLN.2123A	0	1.1	1.9	20
CLN.2460E	0.2	2.9	3.3	11.3
CLN.2468A	0.2	2.8	3.7	15.8
CLN.2498E	0.4	2.1	2.7	11.9
CLN.2545A	0.2	1.2	1.9	14.9
CLN.2545B	0.1	1.5	2	17.1
F1.3019.Galina	0	0.4	0.4	68.1
Favi.9	0	0	0	53.7
FTC.6231	0	0	0	46.7
FTC.6236	0	0	0	28.1
FTC.7088	0.1	0.2	0.2	35
FTC.7127	0.1	2.5	2.5	13.6
FTC.7351	0.1	1.9	2.3	12.1
FTC.7483	0	1.6	1.6	11.9
GemPride	0	0.1	0.1	19.2
HA.3060	0.1	0.1	0.1	22.1
HMX.4810	0.1	0.3	0.3	56
Industry.DR.10403	0	0.3	0.3	41.7
Nadira	0	0	0.2	36
Nirouz.TH.99806	0	0.2	0.2	37.3
O4.108	0.1	0.3	0.3	43.7
O4.240	0	0.3	0.5	34.2
O4.495	0	0.3	0.3	52
O4.498	0	0.6	0.8	65.1
O4.501	0.2	0.9	1.2	27.6
Ponchita	0	0	0	26.6
PS.43316	0	1.2	1.1	11.4
PT.4722A	0.2	1.5	1.8	19.6
Realeza	0	0	0	47.4
Roma.VF	0.1	3	2.9	20.3
Sasya.0202.F1	0	0.6	0.6	40.9
Thoriya	0	0	0	35.9

TLCV.15	0.1	2.3	3	26.2
TY.75	0	0	0	53.4
Xina	0	2	3.2	12.4
Yassamen.TH.99802	0	0	0	51.5
Yosra	0	0	0	54.1

Year 1 Preliminary Trial – Lomé, Togo

Variety	Sev1	Sev2	Sev3
Atak	0	0.9	2.7
Bybal	0	1.3	3.2
Chenoa	0	1.5	3
Cheyenne.E448	0	1	2.1
CLN.2123A	0	2.7	2.9
CLN.2460E	0	2.1	2.9
CLN.2468A	0	3.2	3.4
CLN.2498E	0.1	2.5	3
CLN.2545A	0.1	2.2	3.3
CLN.2545B	0.1	2.5	3.6
F1.3019.Galina	0	2.4	3.3
Favi.9	0	2	3
FTC.6236	0	2.4	3.3
FTC.7088	0	2	3.2
FTC.7127	0	2	3.2
FTC.7351	0	3	3.1
FTC.7483	0	2.8	3.2
GemPride	0	2	3.4
HA.3060	0	2.3	3
HMX.4810	0	1.8	3
Industry.DR.10403	0	0.9	1.2
Lety.F1	0	0.4	0.6
Nadira	0	1.7	2.1
Nirouz.TH.99806	0.2	2.5	3.4
O4.108	0	1.7	2.7
O4.240	0.2	1.8	3
O4.495	0.1	1.9	3
O4.498	0.1	2.3	3.2
O4.501	0	2.6	3.1
Ponchita	0	2.4	3
PS.43316	0	1.9	3.2
PT.4722A	0.3	2.7	3.3
Realeza	0	1.1	2
Roma.VF	0	2.4	3.1
Sasya.0202.F1	0	1.1	2
Thoriya	0	1.1	1.6

TLCV.15	0	2.1	3
TY.75	0	2.6	3.4
Yassamen.TH.99802	0.2	2.2	3
Yosra	0	2.4	3.6

Year 2 Advanced Trial – Kargui, Benin

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Yosra	0	0	0	45.5[a]	46.1[a]
Industry.DR.10403	0	0	0	38.7[ab]	37.9[ab]
Realeza	0	0	0	35.8[abc]	35.4[abc]
Atak	0	0	0	33.4[abcd]	32.6[abc]
Nadira	0	0	0	33.2[abcd]	32.8[abc]
Gempride	0	0	0	32.4[abcd]	30.9[abc]
FTC.7127	0	0	0	31.4[abcd]	30.6[abc]
Ponchita	0	0	0	30.0[abcd]	29.0[abc]
Chenoa	0	0	0	29.6[abcd]	28.6[abc]
Thoriya	0	0	0	27.1[abcd]	25.8[bc]
Bybal	0	0	0	26.1[bcd]	25.8[bc]
TLCV.15	0	0	0	23.9[bcd]	23.1[bc]
Lety.F1	0	0	0	22.6[cd]	21.9[bc]
Roma.VF	0	2	3.2	19.4[d]	19.1[c]

Variety	Weight (g)	Length (mm)	Diameter (mm)	Fruits Per Plant
Atak	143.2	46.5	61.4	44.9
Bybal	192.4	54.8	68.4	31.3
Chenoa	102.5	48.9	56.1	35.6
FTC.7127	99.8	55.8	48.3	61.0
Gempride	80.7	48.5	56.2	81.8
Industry.DR.10403	130.2	54.4	59.2	45.8
Lety.F1	20.9	27.6	31.2	107.8
Nadira	131.1	53.4	56.2	55.9
Ponchita	85.1	43.8	58.7	33.3
Realeza	96.6	61.2	44.6	61.8
Roma.VF	75.4	55.4	37.1	44.6
Thoriya	68.1	57.5	46.8	34.6
TLCV.15	122.9	49.9	52.3	61.8
Yosra	144.9	48.4	62.5	53.3

Year 2 Advanced Trial – Kou Valley, Burkina Faso

Variety	Sev1	Sev2	Sev3	Total Yield	Fruits Per Plant
Industry.DR.10403	0.0	2.3	2.7	23.4[a]	45.2
Nirouz.TH.99806	0.0	1.7	2.4	21.0a[b]	29.3
HA.3060	0.1	1.6	2.7	20.4[abc]	23.3
HMX.4810	0.0	1.9	2.6	19.5[abc]	24.1
FTC.6236	0.0	1.9	2.5	19.4[abc]	28.8
Atak	0.0	0.5	2.2	14.2[abc]	28.2
Gempride	0.0	2.1	2.8	13.8[abc]	31.7
Yassamen.TH.99802	0.0	1.1	2.5	12.8[abc]	15.6
Bybal	0.0	0.8	2.4	12.6[abc]	20.7
Thoriya	0.0	2.1	3.1	12.1[abc]	33.2
Cheyenne.E448	0.0	1.6	2.6	10.6[abc]	17.9
Realeza	0.1	2.3	3.0	10.2[abc]	25.4
Ponchita	0.0	1.0	2.5	8.3[abc]	21.7
Lety.F1	0.0	1.8	2.8	7.7[abc]	76.5
Yosra	0.0	1.0	2.4	6.7[abc]	17.2
Chenoa	0.0	1.3	2.5	5.5[bc]	21.3
Roma.VF	0.2	3.0	4.0	4.4[c]	18.3

Year 2 Advanced Trial – Navrongo, Ghana

Variety	Sev1	Sev2	Sev3	Total Yield[NS]	Marketable Yield[NS]
Atak	0.0	0.1	2.1	30.1	19.4
Bybal	0.0	0.1	1.0	26.4	15.2
Chenoa	0.0	0.1	1.9	22.5	16.3
FTC.6236	0.0	0.6	1.5	40.2	32.0
FTC.7351	0.0	1.8	3.2	18.8	9.9
Gempride	0.0	1.0	1.5	27.2	15.6
Industry.DR.10403	0.0	0.8	1.2	31.0	16.7
Lety.F1	0.0	0.4	0.8	19.3	11.0
Ponchita	0.0	0.0	1.3	22.4	13.6
Realeza	0.0	0.7	2.5	27.5	18.6
Roma.VF	0.6	2.1	3.7	24.0	15.6
Thoriya	0.0	0.0	1.2	29.9	19.2
Yosra	0.0	0.4	3.5	20.7	11.0

Variety	Weight (g)	Length (mm)	Diameter (mm)
Atak	101.2	58.3	63.3
Bybal	126.9	63.6	68.6
Chenoa	83.7	56.9	59.8
FTC.6236	89.7	65.6	60.2
FTC.7351	121.1	64.4	70.2
Gempride	85.1	58.8	54.9
Industry.DR.10403	88.1	65.7	60.8
Lety.F1	26.8	41.2	41.4
Ponchita	89.0	53.1	61.0
Realeza	75.8	71.9	53.0
Roma.VF	50.1	65.1	43.0
Thoriya	53.1	59.4	47.1
Yosra	97.1	57.7	66.2

Year 2 Advanced Trial – Baguineda, Mali

(replicate data not provided – no statistical analysis performed)

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Atak	0	0	0	41.2	38.9
Bybal	0	0.8	1	34.7	32.9
Chenoa	0	0.3	0.4	38.8	36.5
Cheyenne.E448	0.7	2.6	2.7	44.7	41.3
Gempride	0.4	1.1	1.2	40.6	37.1
HA.3060	0.3	1.9	2.2	34.4	28.6
HMX.4810	0.3	2.3	2.6	42.8	38
Industry.DR.10403	1.1	2	2.1	42.9	40.5
Lety.F1	0	0.5	0.5	25.5	25.2
Ponchita	0.1	0.6	0.6	40.8	39.4
Realeza	0	0.2	0.3	43.2	41.4
Thoriya	0	0.9	1.1	44.1	42.3
Yosra	0	0	0	42.2	41.4
Roma.VF	2	3.6	4	35.4	34.7

Variety	Weight (g)	Length (mm)	Diameter (mm)	Fruits Per Plant
Atak	108.7	42	53	20
Bybal	156.0	48	56	9
Chenoa	107.3	43	50	14
Cheyenne.E448	87.3	51	69	14
Gempride	102.7	47	46	29
HA.3060	154.0	48	60	18
HMX.4810	154.7	52	65	20
Industry.DR.10403	98.7	54	50	28
Lety.F1	12.0	28	34	30
Ponchita	112.0	43	52	12
Realeza	74.0	55	42	19
Thoriya	59.3	53	44	16
Yosra	184.7	41	51	17
Roma.VF	42.7	56	35	23

Year 2 Advanced Trial – Samanko, Mali

Variety	Sev1	Sev2	Sev3	Total Yield[NS]	Marketable Yield[NS]
Atak	0.0	0.0	0.0	30.5	7.0
Bybal	0.0	0.0	0.0	24.6	6.5
Chenoa	0.0	0.0	0.1	21.8	5.0
CLN.2764-99-13-18	0.0	0.7	0.3	27.6	5.9
FTC.6236	0.1	0.7	0.3	31.7	6.0
Gempride	0.0	0.6	0.1	33.1	8.9
HMX.4810	0.0	0.3	0.1	36.4	8.9
Industry.DR.10403	0.0	0.3	0.4	29.4	7.0
Lety.F1	0.0	0.0	0.0	19.5	5.8
Nirouz.TH.99806	0.0	0.0	0.0	24.6	4.5
Ponchita	0.0	0.0	0.0	26.2	7.9
Realeza	0.0	0.4	0.1	21.0	5.6
Roma.VF	0.2	1.3	3.3	39.6	9.6
Thoriya	0.0	0.0	0.0	26.5	7.0
Yosra	0.0	0.1	0.1	27.8	7.0

Year 2 Advanced Trial – Birni N'Konni, Niger

Variety	Total Yield[NS]	Marketable Yield[NS]	Weight (g)	Length (mm)	Diameter (mm)
Atak	58.4	50.7	77.3	38	45
Bybal	52.4	43.7	108.1	41	44
Chenoa	45.9	41.9	68.6	44	42
Gempride	54.4	50	55	40	38
Industry.DR.10403	43.7	37.5	83.3	44	40
Lety.F1	33	27.9	12.3	21	20
Ponchita	56.3	49.8	71.3	38	42
Realeza	56.7	53	50.9	46	31
Thoriya	54.1	48.4	53.8	44	42
Yosra	56.7	49.5	102.2	42	44
Roma.local	42.4	40	43	43	28
Roma.VF	39.1	37.6	40.9	49	18

Year 2 Advanced Trial – Rufisque, Senegal

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Roma.VF*	1.3	2.8	3.7	55.9[a]	38.4[a]
Ponchita	0.0	0.0	0.0	33.1[ab]	26.4[ab]
TY.75*	0.0	0.0	0.0	44.9[ab]	24.5[ab]
Realeza	0.0	0.0	0.0	38.4[ab]	24.1[ab]
Atak	0.0	0.0	0.0	38.0[ab]	24.0[ab]
Yosra	0.0	0.0	0.0	36.6[ab]	23.6[ab]
FTC.6236	0.0	0.0	0.0	36.1[ab]	22.3[ab]
Chenoa	0.0	0.0	0.0	31.0[b]	21.1[b]
Industry.DR.10403	0.0	0.0	0.0	34.6[ab]	18.6[b]
Lety.F1	0.0	0.0	0.0	36.4[ab]	18.6[b]
Bybal	0.0	0.0	0.0	35.5[ab]	18.4[b]
Thoriya	0.0	0.0	0.0	30.1[bc]	17.8[b]
Gempride	0.0	0.0	0.0	30.9[bc]	17.1[b]
Xina	0.8	2.2	4.0	17.9[c]	8.3[c]

* Note – A discrepancy between data and summary tables makes measurements for these two varieties potentially invalid

Variety	Weight (g)	Length (mm)	Diameter (mm)	Fruits Per Plant
Atak	93.1	41.6	59.0	38.0
Bybal	117.1	47.7	61.7	24.6
Chenoa	85.8	45.0	55.6	26.2
Gempride	66.9	47.5	50.0	81.8
Industry.DR.10403	72.9	44.9	50.4	41.6
Lety.F1	112.2	54.9	58.5	105.2
Ponchita	15.5	26.8	30.2	37.2
Realeza	90.5	43.1	56.8	68.3
Roma.VF	115.2	46.4	61.8	66.6
Thoriya	47.2	56.8	38.0	53.9
Yosra	52.8	46.5	42.8	28.2

Year 2 Advanced Trial – Dapaong, Togo

Variety	Sev1	Sev2	Sev3	Total Yield[NS]
Industry DR 10403	0.0	0.0	0.3	46.0
Bybal	0.0	0.3	1.3	38.3
Chenoa	0.0	0.0	0.3	38.1
Yosra	0.3	0.7	1.1	37.1
Yassamen TH 99802	0.7	0.8	1.4	35.6
Thoriya	0.0	0.0	0.7	35.6
HA 3060	0.0	1.2	1.7	34.9
Gempride	0.0	1.0	1.3	33.6
Atak	0.3	0.3	0.3	32.4
Nirouz TH 99806	0.3	0.3	0.8	32.2
Cheyenne E448	0.8	0.7	1.4	29.9
Ponchita	0.0	0.0	0.0	29.4
Lety F1	0.3	0.7	0.7	22.4
Roma VF	0.3	1.1	2.0	20.4
Realeza	0.0	0.0	0.3	20.2

Year 3 Multi-Location Trial: Kargui, Benin

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Realeza	0.0	0.1	0.4	31.4[a]	26.0
Thoriya	0.0	0.1	0.3	27.5[ab]	22.7
Atak	0.0	0.2	0.5	23.4[abc]	19.7
Gempride	0.1	0.3	0.5	17.5[abc]	13.3
Bybal	0.1	0.3	0.6	16.9[abc]	13.1
Yosra	0.1	0.3	0.6	15.3[bc]	12.3
Roma VF	0.1	1.7	3.3	11.3[c]	9.1

Year 3 Multi-Location Trial: Tombotou, Benin

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Atak		0	0.8	13.6	12.1
Thoriya		0	0.4	10.4	9.3
Realeza		0	0.4	9.7	8.0
Gempride		0	1.4	7.8	6.6
Yosra		0	0.6	7.4	5.6
Bybal		0	0.5	6.1	4.3
Roma VF		0	2.8	2.1	1.2

Year 3 Multi-Location Trial – Navrongo, Ghana

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Yosra	0.0	0.4	0.9	44.2[a]	38.3[a]
Bybal	0.0	0.6	1.7	40.9[a]	32.9[b]
Atak	0.0	0.6	1.3	34.1[b]	26.4[c]
Gempride	0.0	1.0	1.8	27.6[c]	22.4[c]
Lety F1	0.0	0.2	0.6	20.6[d]	17.2[d]
Roma VF	0.5	1.5	3.2	9.9[e]	6.4[e]

Year 3 Multi-Location Trial – Techimantia, Ghana

Variety	Sev1	Sev2	Sev3
Lety F1	0.0	0.2	0.8
Yosra	0.0	0.6	1.0
Bybal	0.0	0.8	1.4
Atak	0.0	1.0	1.7
Gempride	0.0	1.3	2.0
Roma VF	0.6	1.7	3.7

Year 3 Multi-Location Trial – Djakorba, Mali

Variety	Sev1	Sev2	Sev3	Total Yield
Bybal	0		0	1.2
Gempride	0.1		1.8	0.2
Atak	0		0	1.1
HMX 4810	0		0.8	0.6
Ponchita	0		0	0.7
Roma VF	0.4		3.7	0.6

Year 3 Multi-Location Trial – Kollo, Niger

Variety	Total Yield^{NS}	Marketable Yield^{NS}
Bybal	23.9	20.3
Atak	19.9	17.6
Ponchita	17.9	16.3
Yosra	11.9	11.2
Gempride	7.4	6.4
Roma VF	6.9	6.2

Year 3 Multi-Location Trial – Birni N'Konni, Niger

Variety	Total Yield	Marketable Yield
Yosra	58.8^{a}	56.2^{a}
Ponchita	46.4^{b}	44.3^{b}
Atak	45.1^{b}	42.2^{b}
Bybal	44.4^{b}	43.5^{b}
Gempride	23.8^{c}	21.7^{c}
Roma VF	15.0^{c}	13.0^{c}

Year 3 Multi-Location Trial – Dapaong, Togo

Variety	Sev1	Sev2	Sev3	Total Yield[NS]	Marketable Yield[NS]
Atak	0.7	1.2	2.2	13.3	12.5
Thoriya	0.7	1.5	2.1	11.9	11.3
Yosra	0.3	1.2	2.3	9.5	9.1
Bybal	0.8	1.2	1.7	4.5	4.2
Gempride	0.6	1.0	2.1	3.9	3.8
Roma VF	0.8	2.1	2.8	3.9	3.6
HA 3060	0.8	1.4	1.9	3.2	3.1

Year 3 Multi-Location Trial – Kara, Togo

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Thoriya	0.3	1.4	2.3	28.9[a]	21.3[a]
Gempride	0.5	1.4	2.1	20.3[ab]	15.7[ab]
HA 3060	0.6	1.8	2.9	18.2[ab]	13.0[ab]
Roma VF	0.9	2.4	3.4	13.9[b]	12.3[ab]
Yosra	0.6	1.3	2.7	12.9[b]	9.8[b]
Bybal	0.4	1.7	2.9	10.6[b]	8.0[b]
Atak	0.3	2.0	3.3	10.0[b]	8.8[b]

Year 2 Preliminary Trial – Kargui, Benin

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
FTC 6236	0.0	0.0	0.0	39.5	39.1
Llanero	0.0	0.0	0.0	37.3	36.7
HA 3060	0.0	0.0	0.0	32.6	32.2
HA 3074	0.0	2.0	3.0	32.1	31.5
Bwth CO12	0.0	0.0	0.0	31.7	31.3
HMX 4810	0.0	0.0	0.0	31.5	30.9
BWTH CO03	0.0	0.0	0.0	30.3	29.9
Industry DR 10401	0.0	0.0	0.0	29.8	29.3
F1 641	0.0	0.0	0.0	29.5	29.0
HA 3019	0.0	0.0	0.0	28.3	28.0
Porfyra F1	0.0	0.0	0.0	27.1	26.6
NUN 5025 TO	0.0	0.0	0.0	26.0	25.6
MT 158	0.0	0.0	0.0	26.0	25.7
Hamoud Mumyes	0.0	0.0	0.0	25.2	24.8
DRW 7215 F1	0.0	0.0	0.0	25.0	24.7
F1 Savana	0.0	0.0	0.0	24.9	24.6
Gem Pear	0.0	0.0	0.0	24.7	24.3
Dennolino F1	0.0	0.0	0.0	24.6	24.2
F1 1494	0.0	0.0	0.0	24.5	24.3
CLN 2545B	0.0	0.0	0.0	24.5	23.8
Nirouz TH 99806	0.0	0.0	0.0	23.3	22.8
Bwth CO17	0.0	0.0	0.0	23.3	22.7
Espadilha	0.0	0.0	0.0	23.2	22.8
Aegean	0.0	0.0	0.0	23.0	22.7
Setcopa	0.0	0.0	0.0	22.9	22.6
Athyla F1	0.0	0.0	0.0	22.5	22.1
Gem Pack	0.0	1.5	3.0	22.2	21.9
TY 75	0.0	0.0	0.0	22.0	21.6
PS 43316	0.0	0.0	0.0	21.3	20.9
F1 Veuona 483	0.0	0.5	2.2	21.3	20.9
Mrutunjanya	0.0	0.2	3.0	20.9	20.3
F1 Floradida 495	0.0	2.0	2.0	20.7	20.0
Sensei	0.0	0.0	0.0	19.5	19.1
Valor F1	0.0	2.0	3.0	19.5	19.0
Roma VF	1.0	2.0	3.5	10.0	9.9

Year 2 Preliminary Trial – Kou Valley, Burkina Faso

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Aegean	0.0	0.4	2.3	50.2	50.0
F1 Savana	0.1	0.5	2.3	20.1	18.8
Gem Pear	0.1	1.6	2.8	11.9	9.8
Sensei	0.0	0.0	1.4	11.8	11.0
F1 Floradida 495	0.1	1.8	3.0	10.3	10.0
Setcopa	0.2	0.4	2.3	9.2	8.1
Espadilha	0.0	0.7	2.8	9.0	7.8
Mrutunjanya	0.4	2.3	3.0	9.0	7.7
Nirouz TH 99806	0.3	1.1	2.8	7.7	6.6
F1 1494	0.0	0.0	1.3	7.6	6.8
HA 3019	0.0	0.8	2.7	7.2	6.7
Dennolino F1	0.0	0.4	1.3	6.1	5.2
HA 3074	0.1	0.5	2.8	5.7	5.2
F1 Veuona 483	0.0	2.0	3.0	5.0	4.6
Hamoud Mumyes	0.0	0.3	2.7	4.9	4.3
Valor F1	0.0	0.3	2.9	4.6	4.4
Nun 5025 TO	0.0	0.0	0.9	4.4	4.2
Industry DR 10401	0.0	1.2	2.9	3.7	2.9
MT 158	0.1	1.1	2.9	3.6	3.0
BWTH CO12	0.2	2.5	3.4	3.4	2.8
Gem Pack	0.1	2.0	3.0	3.2	2.3
Porfyra F1	0.0	0.1	2.2	3.0	2.9
DRW 7215 F1	0.3	0.3	1.4	2.9	2.5
Athyla F1	0.0	0.3	2.7	2.6	2.1
F1 641	0.0	0.4	3.0	2.5	2.4
Roma VF	0.2	3.0	4.0	1.9	1.5
Llanero	0.0	1.5	3.0	1.5	1.4
BWTH CO03	0.2	1.1	3.0	1.3	1.3
BWTH CO17	0.5	0.0	3.5	1.3	1.1

Year 2 Preliminary Trial – Navrongo, Ghana

Variety	Sev1	Sev2
Industry DR 10401	0.3	1.4
Dennolino F1	0.3	1.5
DRW 7215 F1	0.2	1.5
F1 1494	0.4	2.0
F1 Savana	0.7	2.0
Gem Pack	1.5	2.0
Gem Pear	1.3	2.3
Sensei	0.1	2.4
BWTH CO12	1.4	2.5
F1 641	0.8	2.5
Mrutunjanya	0.4	2.5
Athyla F1	1.7	2.6
BWTH CO03	1.6	2.6
F1 Veuona 483	1.5	2.6
HA 3074	1.5	2.7
NUN 5025 TO	0.5	2.7
Roma VF (local Ghana)	1.5	2.7
Valor F1	1.1	2.7
BWTH CO17	1.2	2.8
Hamoud Mumyes	1.3	2.8
HA 3019	1.5	2.9
Llanero	0.9	2.9
Aegean	1.3	3.0
F1 Floradida 495	1.4	3.0
MT 5025 TO	1.2	3.0
Nirouz TH 99806	0.7	3.1
Setcopa	0.5	3.4
Espadilha	0.8	3.5
Porfyra F1	1.2	3.5
Roma VF	1.5	3.6

Year 2 Preliminary Trial – Baguineda, Mali

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Aegean	0.0	0.1	0.8	3.4	3.3
Athyla F1	0.0	0.0	0.7	10.4	10.2
BWTH CO03	0.3	0.9	1.3	15.0	14.2
BWTH CO17	1.0	3.0	3.5		
Dennolino F1	0.0	0.1	0.2	7.0	7.0
DRW 7215 F1	0.1	0.2	0.7	12.2	11.3
Espadilha	0.0	0.0	0.1	10.5	10.3
F1 1494	0.0	0.0	0.0	5.9	5.8
F1 641	0.5	0.8	1.9	27.6	21.6
F1 Floradida 495	0.0	0.0	0.2	12.4	9.8
F1 Savana	0.0	0.0	0.0	33.6	32.3
F1 Veuona 483	0.2	1.0	2.3	15.0	11.9
Favi 9	0.3	0.3	1.7	11.3	9.1
Gem pack	1.1	2.4	3.0	21.3	16.5
Gem pear	0.1	0.6	3.1	12.5	8.4
HA 3019	0.1	0.3	1.2	3.4	2.8
HA 3074	0.1	0.3	1.4	10.8	9.7
Industry DR 10401	0.2	0.7	2.4	18.1	17.8
Llanero	0.0	0.0	3.0		
Mrutunjanya	1.0	1.2	2.3	4.1	3.7
MT 158	0.5	1.0	1.0		
Nirouz TH 99806	0.2	1.0	3.0	2.1	1.8
Nun 5025 TO	0.2	0.2	1.8	26.4	22.3
Porfyra F1	0.1	0.1	0.2	8.3	8.2
Roma VF	1.1	3.0	4.0	9.8	9.7
Sensei	0.0	0.0	0.0		
Setcopa	0.1	0.2	0.8	24.7	24.5
Valor F1	0.0	0.8	2.4	22.8	18.3

Year 2 Preliminary Trial – Samanko, Mali

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
HA 3019		0.4	1.3	49.4	26.5
CLN 3074		0.0	0.7	49.2	27.0
CLN 2768-31-18-6-5		0.3	1.3	47.8	25.7
CLN 3069		1.7	2.3	47.5	22.7
Porfyra F1		0.3	1.2	46.7	30.2
CLN 3076		0.3	1.3	46.4	33.3
CLN 2777-168-27-2-7-17		1.2	2.2	46.3	27.2
CLN 3077		0.1	1.4	45.5	34.4
Llanero		0.3	2.0	44.9	25.9
CLN 2768-69-23-30-30-27		0.9	1.8	43.9	30.5
CLN 3022		0.3	1.7	43.1	33.7
CLN 3078		0.1	1.1	42.6	30.3
BWTH CO17		1.4	1.5	41.1	30.9
FLA 060825-8		1.0	1.0	41.0	31.1
Valor F1		0.8	2.0	39.4	23.2
CLN 3024		0.6	1.1	39.1	25.0
BWTH CO12		2.4	2.9	38.7	29.0
Mrutunjanya		0.3	1.6	38.4	28.9
HA 3074		1.0	1.4	37.5	23.2
BWTH CO03		1.1	1.4	37.4	14.4
Setcopa		0.0	0.0	37.3	27.4
DRW 7215 F1		0.2	0.7	36.4	31.5
Industry DR 10401		0.3	1.4	36.3	22.5
Roma VF		2.1	3.2	36.0	22.7
CLN 3048		0.5	1.6	35.1	21.0
CLN 2777-168-27-2-15		1.0	1.0	34.6	19.5
FLA 060702-Y9		0.8	1.7	34.3	21.5
Gem Pear		1.5	1.6	34.1	6.4
NUN 5025 TO		1.0	1.5	34.0	14.3
FLA 024525-9		0.3	1.0	33.8	19.3
FLA 000595-2		0.0	1.5	33.8	23.3
Aegean		0.0	1.0	32.9	14.1
CLN 2768-31-18-6-7		1.6	1.8	32.5	25.8
F1 Veuona 483		0.9	2.8	32.1	7.3
CLN 2777-168-27-2-7-8		0.3	1.2	32.0	24.2

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Gem Pack		2.2	2.6	30.8	9.1
F1 641		0.6	1.4	30.6	9.3
F1 Savana		0.0	1.8	30.6	8.0
CLN 3021		0.9	2.1	29.1	13.4
Espadilha		0.0	0.5	29.0	21.7
F1 1494		0.0	0.5	28.2	18.8
Nirouz TH 99806		0.7	0.8	28.2	13.2
F1 Floradida 495		0.1	0.9	28.1	5.0
Hamoud Mumyes		0.0	0.5	27.4	19.8
CLN 2460E		0.3	1.8	27.1	20.2
MT 158		1.4	2.4	27.0	5.5
Fla 024652-Y1 (GC 173)		0.0	1.5	26.7	16.9
FLA 060856-YSBK		0.6	0.2	26.1	10.3
Fla 060887-Y10		0.2	0.8	24.2	19.5
Sensei		0.4	0.5	24.0	17.8
Dennolino F1		0.0	0.1	23.0	17.0
Athyla F1		0.0	0.0	22.2	12.3
CLN 2764-99-13-18-10-15		0.8	1.8	20.4	14.8

Year 2 Preliminary Trial – Birni N'Konni, Niger

Variety	Total Yield
F1 1494	49.0
Roma VF	48.4
Athyla F1	48.0
Nadira	46.5
Industry Dr 10401	41.9
Aegean	41.1
Hamoud Mumyes	38.5
Cheyenne E448	36.8
Bwth Co12	35.8
Drw 7215 F1	35.0
F1 Veuona 483	35.0
Espadilha	34.6
Sensei	34.5
Roma VF (local Niger)	34.2
Bwth Co17	33.2
Nirouz Th 99806	31.8
Dennolino F1	31.4
F1 Savana	30.5
Valor F1	30.4
Mt 158	29.9
F1 641	29.2
Setcopa	28.5
Nun 5025 To	23.9
F1 Floradida 495	21.0
Llanero	20.4
Ftc 6236	18.4
Gem Pear	17.7
TY 75	16.3
Gem Pack	16.0
Ha 3074	15.9
Porfyra F1	15.9
BWTH CO03	11.2
Yassamen Th 99802	9.8
Ha 3019	8.3
Hmx 4810	8.0
Ha 3060	6.1
Mrutunjanya	0.8

Year 2 Preliminary Trial – Rufisque, Senegal

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
HMX 4810	0.0	0.3	0.3	65.9	38.4
FTC 7088	0.0	0.1	0.0	62.8	26.1
Industry DR 10401	0.0	0.2	0.3	56.9	31.7
HA 3060	0.0	0.1	0.1	53.8	15.7
Sensei	0.0	0.0	0.0	52.3	34.8
Valor F1	0.0	0.2	0.3	51.6	27.2
FTC 6231	0.0	0.5	0.6	51.4	31.0
Sasya 0202 F1	0.3	0.9	1.3	51.2	36.2
Gem Pack	0.1	0.1	0.5	49.3	23.9
Nirouz TH 99806	0.0	0.1	0.1	47.7	34.8
Gem Pear	0.0	0.2	0.5	47.3	21.7
Mrutunjanya	0.1	0.7	0.8	46.1	38.1
F1 Veuona 483	0.0	0.2	0.4	44.3	21.4
Porfyra F1	0.0	0.0	0.0	44.1	23.4
Favi 9	0.0	0.2	0.3	44.0	24.2
Yassamen TH 99802	0.0	0.0	0.0	43.8	26.0
Roma VF	0.9	2.8	2.9	40.6	29.3
Nadira	0.0	0.0	0.1	40.5	30.2
NUN 5025 TO	0.0	0.0	0.1	40.1	13.8
DRW 7215 F1	0.0	0.0	0.0	39.8	29.1
BWTH CO17	0.6	1.9	2.1	39.7	26.2
HA 3074	0.0	0.0	0.0	39.6	21.5
F1 Floradida 495	0.0	0.0	0.0	39.0	21.1
F1 1494	0.0	0.0	0.0	38.9	31.0
Athyla F1	0.0	0.0	0.0	38.9	20.0
F1 641	0.0	0.3	0.9	38.8	16.2
Llanero	0.0	0.0	0.0	34.8	20.3
HA 3019	0.0	0.0	0.0	31.0	13.6
BWTH CO12	0.5	2.0	2.4	30.0	16.9
MT 158	0.0	0.0	0.0	29.8	15.9
BWTH CO03	0.7	1.4	1.9	29.7	16.0
F1 Savana	0.0	0.0	0.0	27.6	20.1
Dennolino F1	0.0	0.0	0.0	21.5	16.1
Aegean	0.0	0.0	0.0	18.2	10.1

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Setcopa	0.0	0.0	0.0	17.8	13.8
XINA	0.5	1.5	2.1	14.6	11.9
Hamoud Mumyes	0.0	0.0	0.0	13.7	10.4
Espadilha	0.0	0.0	0.0	10.6	7.2

Year 2 Preliminary Trial – Dapaong, Togo

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Industry DR 10401	0.0	0.0	1.0	31.9	29.7
DRW 7215 F1	1.0	1.0	1.0	26.1	24.6
Sensei	0.0	0.0	1.0	26.0	25.8
Athyla F1	1.0	1.0	1.7	23.2	20.4
BWTH CO03	1.5	1.7	2.0	22.5	22.2
BWTH CO17	1.0	1.0	1.0	22.1	17.7
Mrutunjanya	0.0	1.0	1.5	21.6	20.6
F1 1494	0.0	0.0	2.0	21.5	20.0
BWTH CO12	1.0	1.0	1.0	21.0	19.2
Aegean	1.0	2.3	1.8	19.0	15.3
F1 Floradida 495	1.1	1.2	1.6	18.3	17.7
Porfyra F1	0.0	1.0	1.0	18.2	16.8
Espadilha	0.0	0.0	0.0	17.3	15.7
Nirouz TH 99806	0.0	1.0	1.0	17.0	15.7
F1 Savana	0.0	1.0	1.5	16.0	15.3
F1 Veuona 483	1.1	1.3	1.3	16.0	13.7
Roma VF	0.5	1.2	1.4	15.9	11.5
Setcopa	0.0	0.0	0.0	15.5	13.4
F1 641	1.0	1.0	1.0	14.2	14.0
Gem Pack	0.0	1.1	1.1	14.1	13.4
Valor F1	0.0	0.0	1.0	14.0	10.3
HA 3019	0.0	1.6	1.1	13.8	12.7
HA 3074	1.0	1.0	1.5	13.7	12.5
Dennolino F1	0.0	0.0	0.0	13.2	12.2
Gem Pear	1.0	1.2	1.1	11.9	11.3
NUN 5025 TO	0.0	1.0	1.4	11.0	9.8
MT 158	0.0	1.2	1.1	10.0	9.1
Llanero	1.0	1.0	1.0	9.8	8.8
Hamoud Mumyes	0.0	0.0	0.0	9.7	8.2

Year 3 Advanced Trial – Kargui, Benin

Variety	Sev1	Sev2	Sev3	Total Yield[NS]	Marketable Yield
Setcopa	0.1	0.3	0.7	16.4	11.9[a]
Sensei	0.0	0.3	0.6	16.4	11.4[a]
Athyla F1	0.1	0.3	0.6	16.2	10.2[ab]
Dennolino F1	0.1	0.4	0.6	15.5	8.7[ab]
Roma VF	0.4	1.8	3.3	17.2	8.2[ab]
Espadilha	0.1	0.6	1.2	10.3	5.8[b]
Porfyra F1	0.1	0.4	0.9	9.1	4.9[b]

Variety	Weight (g)	Length (mm)	Diam (mm)	Lobes
Porfyra F1	108.1[a]	52.1[b]	57.8[a]	3.9
Athyla F1	85.4[ab]	45.5[bc]	57.3[a]	4.3
Setcopa	82.6[ab]	46.0[bc]	56.0[a]	4.3
Dennolino F1	70.9[bc]	45.6[bc]	49.9[ab]	2.6
Espadilha	70.3[bc]	44.7[bc]	53.7[ab]	3.9
Sensei	62.2[bc]	40.6[c]	46.9[b]	2.7
Roma VF	47.4[c]	65.3[a]	37.3[c]	2.5

Year 3 Advanced Trial – Samanko, Mali

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Dennolino F1	0.0	0.0	0.0	29.5[a]	25.0[a]
Setcopa	0.0	0.0	0.0	29.4[a]	20.5[ab]
Sensei	0.0	0.0	0.0	29.1[a]	22.4[ab]
Espadilha	0.0	0.0	0.0	25.3[ab]	16.6[abc]
Porfyra F1	0.0	0.0	0.0	21.5[ab]	12.8[bc]
Roma VF	0.4	1.8	3.3	15.0[bc]	6.7[c]

Year 3 Advanced Trial – Dapaong, Togo

Variety	Sev1	Sev2	Sev3	Total Yield	Marketable Yield
Setcopa	0.9	1.1	1.8	4.2[a]	4.2[a]
Dennolino F1	1.0	1.2	1.7	3.0[ab]	3.0[ab]
Sensei	0.9	1.1	1.7	2.4[ab]	2.4[ab]
Espadilha	1.0	1.1	1.8	2.2[ab]	2.0[ab]
Athyla F1	1.0	1.1	1.6	2.0[ab]	2.0[ab]
Roma VF	1.1	2.1	2.8	1.8[ab]	1.8[ab]
Porfyra F1	1.2	1.8	2.2	1.0[b]	1.0[b]

APPENDIX 3

GPS COORDINATES OF TRIAL LOCATIONS

Guene, Benin:	11.9323 N	3.23347 E
Tomboutou, Benin:	11.8751 N	3.27585 E
Kou Valley, Burkina Faso:	11.2294 N	4.16361 W
Kumasi, Ghana:	6.68025 N	1.66864 W
Navrongo, Ghana:	10.836 N	1.09864 W
Techimantia, Ghana:	7.21949 N	2.0156 W
Djakorba, Mali:	13.5781 N	5.91255 W
Niono, Mali:	14.2793 N	5.95072 W
Samanko, Mali:	12.5309 N	8.07708 W
Sibby, Mali:	12.3518 N	8.38469 W
Sotuba, Mali:	12.6525 N	7.926 W
Birni N'Konni, Niger:	13.8241 N	5.28861 E
Rufisque, Senegal:	14.7771 N	17.2239 W
Dapaong, Togo:	10.8787 N	0.160417 E
Kara, Togo:	9.3631 N	1.32491 E

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