Plant Biotechnology: Potential Impact for Improving Pest Management in European Agriculture

Tomato – Virus-Resistant Case Study
December 2003

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Introduction

The cultivated tomato originated from wild plants found in the Andean regions of Chile, and Peru. The tomato was first domesticated in Mexico. In the early 16th century, Spanish conquistadors brought the tomato to Europe. The first record of tomatoes in Europe is credited to an Italian herbalist in 1554. The plant and its fruit were slow to gain acceptance in Europe except as an ornamental, a medicinal or a curiosity- tomatoes were often hurled at unsatisfactory show business acts. References to the eating of tomatoes are quite rare. Europeans knew it was related to poisonous members of the nightshade family. Called the "mad apple" or "rage apple", it was also considered a powerful aphrodisiac-the French named it *pomme d'amour*, "apple of love." Grown in 16th century England as an ornamental plant, the tomato was carried by colonists to North America. But it didn't appear in American marketplaces until the 19th century. Thomas Jefferson made frequent references to the culinary uses of tomatoes at Monticello in the early 1800s. One Robert Johnson, who is not otherwise known to history, achieved a degree of celebrity (and advanced the cause of the tomato as a food) by eating a tomato on the steps of the courthouse in Salem, N.J. in 1820. Even after the tomato came to be accepted as a food, debate persisted over whether it is a fruit or vegetable. Botanically it is a fruit, since it develops from an ovary. In 1893, the U.S. Supreme Court ruled that the tomato is a vegetable (because it is usually eaten in a meal when vegetables are eaten) in a case that challenged the payment of tariffs (imported fruits were duty free while vegetables were dutiable).

There are two models for tomato production in the European Union: Holland, Belgium, the U.K., Denmark and Germany primarily use a soilless system in which tomatoes are cultivated in greenhouses on substrates (principally rockwool). In these northern countries, the tomatoes are grown for the fresh market. In Mediterranean countries, tomatoes are grown in soil either in the field or in greenhouses. The four Mediterranean countries (Greece, Italy, Portugal and Spain) produce 15 billion kilograms of tomatoes on 236,000 hectares with a value of €8.7 billion/yr (see Table 1). Approximately 6% of Italy's tomato production occurs in greenhouses [20]. Approximately 65% of the tomatoes grown in the four Mediterranean countries are for processing, while the rest are marketed fresh.

Tomato Yellow Leaf Curl Virus (TYLCV)

Tomato yellow leaf curl virus is a whitefly-transmitted virus first described from infected tomato plants in Israel in the 1960s [10]. Tomato yellow leaf curl disease is caused by viruses belonging to the genus *Begomovirus,* in the family geminiviridae. Viruses in this family, usually referred to as geminiviruses, are characterized by single stranded circular DNA encapsulated in twinned virus particles.

For a successful infection, TYLCV must be delivered into the phloem sieve tubes by the whitefly when feeding. Once inside a tomato cell nucleus, the viral DNA replicates by a rolling circle mechanism via double-stranded DNA intermediate replicative forms [15].

Tomato yellow leaf curl virus symptoms appear several weeks after infection and include severe stunting, marked reduction in leaf size, upward cupping and chlorosis of leaf margins, mottling, flower abscission, and significant yield reduction [1].

A very common European weed, black nightshade, is an excellent reservoir for both the virus and the whitefly. It is possible that tomato yellow leaf curl virus spread from this weed into tomato for the first time in the 1980s.

Due to the serious nature of the disease, the European Council Directive 2000/29/EC prohibits the movement of infected tomato plants into and within the Community. Geminiviruses have not become a problem in the tomato greenhouses of Netherlands or the U.K. Tomato yellow leaf curl virus does not occur in France [4]. Silverleaf whitefly has attained a notifiable pest status in European countries such as the U.K. It is the subject of eradication programs and quarantine legislation.

In Italy, damage from tomato yellow leaf curl virus has been recorded since 1988, when the problem arose in tomato crops (both greenhouse and outdoor) located in Sardinia and Sicily [11]. Initially, the virus occurred only in these areas, but it spread rapidly in Italy and in 1991, symptoms were also recorded in Calabria [11]. In Sicily, the virus has caused huge losses over about five years [11]. In Sicily, netting of glasshouses was not used up to the end of the 1980s, but they were rapidly adopted after the introduction of the tomato yellow leaf curl virus [11].

In Italy, the presence of the whitefly in outdoor conditions seems to be limited to the warmer regions, namely the areas south of 41 degrees N, where the whitefly is probably able to overwinter outdoors. On the other hand, the whitefly is ubiquitous in greenhouse conditions [6]. Surveys suggest that in northern and central Italy, the whitefly can occasionally be found in open fields only close to infested protected crops [6].

Tomato yellow leaf curl virus infections have caused severe yield losses to tomato crops of south and southeastern Spain since they were first reported in 1992 [12]. Since 1992, TYLCV has spread to all of the main vegetable-producing regions of southern Spain where it has become the limiting factor for tomato production during the summer and fall causing up to 100% yield loss [13]. Both greenhouse and field grown tomatoes have been infected [18]. Due to severity of symptoms, many tomato growers have been forced to replant greenhouse crops up to 3 times during the summer months. Around the Malaga region, growers have recently had to abandon the growing of outdoor tomato crops.

In Portugal, tomato yellow leaf curl virus was first reported in 1995 [2]. In the first years of the outbreak, disease incidence was up to 100% in many cases [5]. Since then, virus epidemics have occurred annually, being a limiting factor mainly for the autumn/winter greenhouse tomato crops [2]. The growing area of tomatoes in the Algarve region (south Portugal) has declined almost 48% since 1995, mainly due to the epidemics [3]. Research has shown that screen net protection and weekly insecticide sprays provided improved protection from the whitefly-transmitted disease [5].

In late summer 2000, tomatoes grown in greenhouses in Greece showed symptoms of yellow leaf curl virus. More than 30 hectares of tomato greenhouses were affected and the disease incidence ranged from 15 to 60%, with estimated crop losses of over $500,000 [7]. By 2001, distribution and incidence had increased to the majority of tomatoes grown in greenhouses in Greece [16]. In most cases, the disease incidence was 80 to 90%, or even 100% [16].

Use of chemical insecticides is required for effective control of whitefly because a single adult, in the presence of an infected plant may disseminate the virus to the entire crop as well as to those in the vicinity [9]. The use of chemical insecticides to prevent TYLCV spread by whiteflies is widespread in Europe [17].

Control of TYLCV is currently based on insecticide treatments targeted at the whitefly [15]. Imidacloprid is a highly effective insecticide for control of whiteflies and it has been used intensively since it was introduced into greenhouse crops in the 1990s.

Physical barriers such as 50 mesh screens are routinely used in greenhouses to limit TYLCV transmission by whiteflies in Mediterranean countries. Insect-proof nets have been widely used to protect tomato crops in open field conditions [17].

Regardless of attempts to control TYLCV, all greenhouses have at least a 1% infection rate [14]. Complete eradication or exclusion of whiteflies is impossible.

Approximately 2,500 square meters of netting are used per hectare at a cost of €0.80/square meter [19]. The nets are replaced every 5 to 8 years. Assuming that the nets are replaced every 8 years implies an average annual cost of netting greenhouse tomatoes at €250/ha.

Assuming that 6% of tomato hectares in Greece, Italy, Portugal and Spain are in greenhouses implies a greenhouse area of 14,000 hectares. Assuming that all of these hectares are netted to prevent whitefly intrusion implies an aggregate annual cost of €3.5 million.

Imidacloprid costs approximately $281/lb AI and is used at 0.2lb AI/application [21] [22]. Assuming six applications to all the greenhouse hectares implies a total aggregate use of 16,000 pounds of active ingredient at a cost of $4.7 million.

It is estimated that there are 222,000 hectares of tomatoes planted in open fields in the 4 Mediterranean countries, and that 50% are affected by whitefly infestations resulting in two applications of imidacloprid. These assumptions imply a total use of 44,000 pounds of active ingredient costing $12.4 million.

Assuming that half of the field hectares (111,000) and all of the greenhouse hectares (14,000), which in total represent 53% of the total acres, incur a yield loss of 1% due to

TYLCV implies an aggregate loss of 69 million kilograms of production with a value of $38 million.

In the U.S., geminiviruses first appeared in Florida's tomato acres in 1989. The incidence of infected plants in some fields was as high as 100%. The viruses were estimated to have reduced the value of the 1990-91 Florida tomato crop by 20% [8]. In Florida, geminivirus management is almost entirely dependent on the use of insecticides, in particular the use of imidacloprid. The incidence of the viruses has remained at low levels due to the insecticide use [8].

In the early 1990s, a project to develop genetic resistance to geminiviruses by transforming plants with virus genes was begun at the University of Florida. As a result, tomatoes have been transformed successfully to have resistance to two geminiviruses including TYLCV [22]. The genetically engineered tomatoes in the U.S. remain in the research stage; no applications have been made for their approval.

Italian researchers have successfully transformed tomato plants by inserting a gene of the tomato yellow leaf curl geminivirus by agrobacterium. The resulting tomato plants were resistant to TYLCV when challenged either directly by the virus or by the whitefly vector [23].

It is assumed that the transgenic tomato varieties would be planted on 53% of the four Mediterranean countries tomato hectares and substitute for the current use of insecticides and insect netting while also eliminating the current loss of 1% of production.

<u>Cucumber Mosaic Virus</u>

The Campania region in Italy has 6,500 hectares of tomatoes grown for processing, which represents 7% of Italy's total tomato area. The region produces 374 million kg of tomatoes for processing, which represents 6% of Italy's total production [24]. Assuming that the region's tomatoes represent 7% of the national value of tomato production implies an aggregate value of €246 million.

The predominant tomato grown for processing in the Campania region is the San Marzano variety. In the Campania region, the traditional variety has been awarded the protected origin label "Pomodoro San Marzano Dell'Agro Sarnese Nocerino". The San Marzano is thin and pointed. The conjecture is that this tomato resulted from a spontaneous hybridization of two other varieties and was distinct from both of them and from the Roma plum tomato best known in the U.S. [25]. According to Neapolitan tradition, pizza was invented as a vehicle for the consumption of the San Marzano. Ash from the eruption of Mt. Vesuvius created a soil rich in potassium and other minerals, which is not found anywhere else on earth [26]. The pulp has a distinctive taste: it has less sugar than other tomato varieties.

Tomato production in the Campania region has declined significantly in the last decade due to epidemics of cucumber mosaic virus. In the 1980s, the region was the number one producing region in Italy. Now, it is 4th or 5th [26]. Campania produced 35% of the San Marzano's grown in Italy. Today it produces 3% [27]. Production in Campania is declining at about 12-16% a year [27]. The concern is that the San Marzano tomato will disappear from the Campania region [28].

Tomato plants affected with cucumber mosaic in the early stages are yellow, bushy and considerably stunted [33]. The most characteristic symptom is shoestring-like leaf blades. Severely affected plants produce few fruit, which are usually small, with delayed maturity [33]. More than 60 aphid species are capable of transmitting the virus. The infected plants become tall, stringy, and thin, making it hard for them to soak up water and impossible to protect the fruit from sun [26].

Different approaches are currently under way to find suitable control measures for CMV. Efforts using traditional breeding for CMV resistance have mostly been unsuccessful [29]. Control of the CMV aphid vectors by insecticides has not been completely effective in preventing virus infections and epidemics [29].

In 1992, collaboration was initiated between the Italian Institute for Plant Pathology and Monsanto in order to produce transgenic tomatoes with resistance to CMV [30]. Field tests were begun in 1993 and lasted through 1999. No insecticides were applied to ensure a large aphid population [30]. In 1997, comparisons were made between the best transgenic lines (four lines were completely virus free) and the conventional lines (90% disease incidence in 10 weeks) [30]. The yield of the transgenic lines was 66% greater than the conventional lines [30]. Research confirmed that the agronomic traits of the transgenic varieties met industry standards for processed tomatoes [34]. Subsequently, the researchers processed and canned the transgenic tomatoes in order to develop a protocol for detecting the foreign genes in food. The method resulted in a very sensitive test, which allowed the detection of transgenes in processed tomato [31]. The researchers also tested for pollen flow between the transgenic plants and untransformed controls in two Italian growing regions [32]. No transgenic flow was detected in the two fields [32].

It is estimated that the planting of the transgenic tomato varieties resistant to CMV could prevent the total loss of the production of San Marzano tomatoes in Campania.

Table 1a: Tomato Production			
	Area (000 HA)	***Production (billion kg)***	***Value (€ billion)***
Greece	36	1.9	0.70
Italy	124	6.4	3.52
Portugal	14	1.0	0.47
Spain	62	3.7	2.52
Total E.U.	245	14.9	8.72
U.S.	50	1.6	1.06

Source: Eurostats

Table 1b: Tomato Production			
	Area (000 A)	***Production (billion Lbs)***	***Value ($ billion)***
Greece	89	4.2	0.70
Italy	306	14.0	3.52
Portugal	35	2.2	0.47
Spain	153	8.2	2.52
Total E.U.	605	32.9	8.72
U.S.	124	3.5	1.06

Dollars and Euros assumed equivalent.

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