Occurrence of *Macrophomina phaseolina* in Israel: Challenges for Disease Management and Crop Germplasm Enhancement

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Abstract

Macrophomina phaseolina is a soil-borne fungal pathogen infecting many important crop plants. The fungus, which can survive on crop debris for a long period of time, causes charcoal rot disease by secreting a diverse array of cell-wall degrading enzymes and toxins. *M. phaseolina* thrives during periods of high temperatures and arid conditions, as typically occur in Israel and other countries with a Mediterranean climate. Crop losses due to charcoal rot can be expected to increase and spread to other countries in a warming global climate. Management of this pathogen is challenging, requiring an array of approaches for the various crop hosts. Approaches that have had some success in Israel include grafting of melons and watermelons on resistant squash rootstocks and soil application of fungicide to reduce disease incidence in melons, fumigation and alterations in planting date and mulching of strawberries, and alteration in irrigation regime of cotton. Elsewhere, these approaches, as well as soil amendments and addition of organisms

that are antagonistic to *M. phaseolina*, have had success in some crop situations. Management through host resistance would be the most sustainable approach, but it requires identifying a resistant germplasm for each crop and introgressing the resistance into the leading cultivars. Resistance to charcoal rot is under complex genetic control in most crops, posing a great challenge for its introgression into elite germplasm. Moreover, fast, reliable methods of screening for resistance would have to be developed for each crop. The toothpick-inoculation method used by us holds great promise for selecting resistant germplasm for melons and possibly for sesame, but other methodologies have to be devised for each individual crop.

Keywords: *Macrophomina phaseolina*, melon, watermelon, cotton, strawberry, sesame, resistance, breeding, germplasm enhancement, global warming

Macrophomina phaseolina is a soil-borne, plant pathogenic fungus that causes a variety of diseases in more than 500 plant species, including numerous economically important warm-season crops such as maize, sorghum, chickpea, common bean, cotton, jute, soybean, sesame, and sunflower (Kaur et al. 2012; Marquez et al. 2021; Saleh et al. 2010; Singh et al. 1990). The fungus causes “charcoal rot,” producing either microsclerotia or pycnidia in the roots and stems of the host, which enable it to survive in crop debris and the soil for prolonged periods (Gupta et al. 2012; Romero Luna et al. 2017). It secretes a diverse arsenal of cell-wall degrading enzymes (Islam et al. 2012; Knogge 1996) as well as toxins (Bhattacharya et al. 1992; Dhar et al. 1982; Kitahara et al. 1991; Mahato et al. 1987), enabling it to penetrate plant cells and destroy host tissues. Plants having lower stem tissues that are soft, such as melon and strawberry, succumb to the cell-wall degrading enzymes leading to tissue rotting, whereas plants having hard, compact lower stems, such as

watermelon, sesame, and cotton, succumb to the toxins, leading to wilting without visible rotting (Fig. 1).

Symptoms of charcoal rot are often absent during the cool, early part of the growing season. As the weather warms and the crop matures to flowering, fruit set, and ever-increasing fruit load, the symptoms appear and are exacerbated if the plants are stressed by heat or drought (Pickel et al. 2020; Reuveni et al. 1982; Romero Luna et al. 2017). *Macrophomina* thrives in regions with prolonged dry periods and elevated temperatures (Marquez et al. 2021; Saleh et al. 2010). Moreover, the ban on use of the soil fumigant methyl bromide has been coincident with a surge of losses in various crops due to charcoal rot. For example, methyl bromide fumigation allowed strawberry growers the world over to enjoy years of freedom from soil-borne pathogens (Chamorro et al. 2016; Holmes et al. 2020). Since the phase-out of this fumigant, *Macrophomina* has become a severe problem in many strawberry-producing countries, including Israel (Pickel et al. 2020; Zveibil et al. 2012).

Israel has a typical Mediterranean climate characterized by long, hot, rainless summers and relatively short, cool, rainy winters (Nevo et al. 2012). This climate favors *Macrophomina*, allowing it to attack a wide range of crops, most notably melon, watermelon, strawberry, and cotton, resulting in considerable yield reduction and economic losses (Omar et al. 2007; Reuveni et al. 1982; Zveibil and Freeman 2005). Although detailed evidence is not available, there is a consensus

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that *Macrophomina* is becoming more important in Israeli agriculture. The number of plant species from which this pathogen was isolated increased from five in 2015 to 23 in 2019. This includes a wide range of horticultural crops, encompassing vegetables, annual and perennial flowers, herbs, lawn, and fruit tree species (G. Elkind, Plant Pathogen Diagnostic Laboratory, Israel Ministry of Agriculture, unpublished data). Over the past 20 years, *Macrophomina* has become the most common fungus isolated from roots of wilted watermelon and strawberry plants cultivated in central and northern Israel (Cohen et al. 2002; Pickel et al. 2020).

Global warming can be expected to increase the occurrence, prevalence, and severity of crop-plant diseases caused by *M. phaseolina* in many growing areas. Global warming could also favor the diffusion of *Macrophomina* from traditional growing areas to new ones (Pandey and Basandrai 2021). The situation in Israel could be an early warning or case study for what other areas of the world could experience in a future, warmer climate. Over the past decade, we investigated the interaction between *Macrophomina* and melon, watermelon, strawberry, sesame, and cotton in Israel. Our objective here is to describe the main obstacles and difficulties we faced in managing this versatile pathogen and learning more about its interaction with these crops, the variation in disease appearance and severity, and the approaches we used for each host-pathogen interaction, to develop guidelines to facilitate research, breeding, and management of this pathogen. In addition, we offer methodologies and discuss prospects for germplasm enhancement and breeding of four of these crops for resistance to *Macrophomina*.

Disease Management

Management of charcoal rot disease caused by *Macrophomina* is challenging, requiring an array of approaches for different hosts under various locations and varying environmental conditions. As *Macrophomina* is highly affected by extreme growing conditions, reducing environmental stresses affecting the plants appears to be a 'common thread' for all the hosts. Nevertheless, each host has its own special settings and requirements regarding appropriate strategies for reducing the effect of environmental stress and disease damage.

Fig. 1. Differences in reaction to *Macrophomina phaseolina* by four crop hosts. **A.** Lesion development in melon. **B.** Internal rotting in strawberry. **C.** Wilting in sesame. **D.** Chlorosis and wilting in cotton.

Crop rotation, at least in Israel, is not effective because most summer crops such as melon, watermelon, chickpea, and sunflower are susceptible to the disease and produce vast amounts of microsclerotia, endangering the following crop. This same situation may occur in other countries and areas having a similar Mediterranean climate. Other cultural methods, such as planning appropriate planting dates and careful irrigation, have proved to be beneficial in some cases elsewhere (Bruton et al. 1987) but cannot always be implemented. Fungicide applications to the soil, though effective for reducing disease incidence and severity in melon, did not affect disease development in cotton growing in Israel (Cohen and Elkabetz 2021; Cohen et al. 2012). Grafting can be used for some horticultural crops and, indeed, grafting melon and watermelon on pumpkin and squash rootstocks is a very effective method for reducing the risk caused by *M. phaseolina* (Cohen et al. 2012, 2014), but there is a need for careful selection of the rootstock-scion combination to avoid negative effects on fruit quality (Condurso et al. 2012; Devi et al. 2020). Management through host resistance would be the most sustainable approach for disease reduction (see below). However, identifying resistant germplasm sources and introgressing resistance into commercially acceptable genetic backgrounds can be quite problematic.

Melon. *Macrophomina* is one of the major causes of wilting disease of melons, *Cucumis melo* L. Also known as vine decline, disease caused by *Macrophomina* can be quite severe in melons (Bruton and Wann 1996). In Israel, this fungus was determined to be the leading cause of melon plant collapse, resulting in a drastic reduction in melon cultivation in the northern Negev Desert of Israel in the late 1970s (Krikun et al. 1982; Reuveni et al. 1982, 1983). Typically, disease symptoms in melons include water-soaked lesions on the main stem, occurring from the soil level acropetally to several centimeters above. Droplets of amber-colored exudate may form, with the lesions eventually turning beige, desiccating, and cracking, with the plants declining and dying prior to harvest (Jacob et al. 2013) (Fig. 1A). To cope with losses occurring from *Macrophomina* in melon crops, fungicide applications and grafting have been deployed (Cohen et al. 2012).

Fungicides used for controlling *Macrophomina* in melon crops include azoxystrobin, alone or combined with chlorothalonil or mefenoxam. Applications have exhibited high efficiency in the control of *Macrophomina* wilt of melons under field conditions. Pyraclostrobin, belonging to the strobilurin group, combined with boscalid, was also effective in a preliminary evaluation (Cohen et al. 2012).

Melon scions of highly desired but susceptible cultivars are often grafted onto pumpkin or squash (*Cucurbita* spp. L.) rootstocks to control soil-borne diseases. Grafting offers a very effective means for disease reduction, which in some cases is accompanied by an increase in yields. The grafted plants cannot be defined as resistant because the pathogen can penetrate the rootstock and, under adverse environmental conditions, cause lesions in the susceptible scion. However, in most cases, a slower rate of pathogen progression within the resistant rootstock roots allows the plant to complete its life cycle. Nevertheless, disease level and yield can vary among years and even within the same year in different fields. Moreover, grafted transplants are expensive to produce, and the extra cost entailed for growing melons in midsummer can be too high to be profitable for the grower (Cohen et al. 2012).

Watermelon. *Macrophomina* is one of the major causal agents of wilting in watermelons, *Citrullus lanatus* (Thunb.) Matsum. & Nakai. Appearance of microsclerotia on the roots and lower stems of dead plants indicates *M. phaseolina* as the likely causal agent of disease (Cohen et al. 2002; Mahdizadeh et al. 2011). Disease symptoms in watermelon are of the "non-specific vine decline" type (Martyn 2008), expressed by partial wilting and reduced foliage accompanied by poorly developed small fruit. The plants wilt shortly before harvest, without the lesions and rotting typically seen in melons (Cohen et al. 2016), which raises questions regarding the mechanisms used by the pathogen to produce disease in these two cucurbit crops.

For some time, *Macrophomina* has been the most common fungus isolated from roots of collapsed watermelon plants in parts of central and northern Israel (Cohen et al. 2002). The involvement of this pathogen in wilting of watermelon has also been reported in Pakistan and Iran (Baloch et al. 2013; Mahdizadeh et al. 2011).

Grafting watermelon scions onto *Cucurbita* rootstocks has been used for general soil-borne disease management in watermelon crops. However, grafting onto such rootstocks can have an adverse impact on fruit quality (Fallik et al. 2016, 2019).

Strawberry. Elimination of the soil fumigant methyl bromide is considered to be the main reason for disease outbreaks in crops of strawberry, *Fragaria × ananassa* Duchesne, in various countries since 2004 (Avilés et al. 2012; Koike 2008; Mertely et al. 2005; Sánchez et al. 2016). In Israel, too, *Macrophomina* was first reported in strawberry parallel to the phase-out of methyl bromide (Zveibil and Freeman 2005; Zveibil et al. 2012). Strawberry is cultivated as a single-season monoculture crop during the winter months in Mediterranean countries, for local fresh-fruit markets and export to Europe and elsewhere. At the end of the growing season, the plant material is usually plowed into the soil, together with any microsclerotia of *Macrophomina* that had infected the plants. The pathogen remains in the soil as sclerotia on plant debris and on dead organic matter throughout the summer months until the following growing season (Zveibil et al. 2012).

Macrophomina in strawberry is favored by high air and soil temperatures (28 to 35°C) (Zveibil et al. 2012). With lack of proper management, charcoal rot can cause as much as 80% plant mortality by the end of the strawberry season (Chamorro et al. 2016). Typical symptoms in strawberry are wilting and collapse of older leaves and subsequent necrosis of the entire plant, similar to symptoms caused by other crown-rot pathogens (Baggio et al. 2021) (Fig. 1B). In warmer climates, charcoal rot symptoms usually occur soon after fall transplanting and again at the end of the season in the spring, when temperatures are relatively high (Baggio et al. 2021).

Preplant treatments for the management of *Macrophomina* in strawberry nurseries and field production plots in Israel consist of timely applications of fumigants (Zveibil et al. 2012). Other techniques may also prove to be useful. For example, later plantings were observed to delay onset of the disease (Freeman et al., unpublished). During the 2019–20 season, the onset of plant mortality in early plantings was approximately 40 days after transplanting whereas plantings made 3 weeks later exhibited mortality 180 days after transplanting. Early in the planting season, soil temperatures are relatively high, favoring *Macrophomina*. Soil temperatures decrease as the autumn progresses, thus creating suboptimal conditions for the pathogen.

Plastic mulches in strawberry cultivation may also reduce the onset of disease. The effect of mulching was tested on two farms in the Sharon Plain of central Israel over a range of planting times. The soil temperature under two colors of plastic mulches was compared, white-on-black and silver-on-black, the latter being more commonly used. The white-on-black mulch resulted in an average 2°C reduction in soil temperature and a delay in the onset of the disease (Freeman et al., unpublished).

Sesame. Sesame, *Sesamum indicum* L., is cultivated mostly in developing countries in warmer regions (Chattopadhyay et al. 2019). Reports of occurrence of charcoal rot of sesame have been made from all over the sesame-growing areas of the world. The incidence of *Macrophomina* is highest during the hottest months, causing stem and root rot, chlorosis, and incomplete capsule filling. *Macrophomina* can be seed-borne in sesame, resulting in seedling mortality and reduced plant stand per unit area. The fungus also affects developing and mature plants, which become ashy in appearance with drooping of the acropetal leaves (Fig. 1C).

Several agrotechniques have been observed to alleviate attacks of *Macrophomina* in sesame (Ibrahim and Abdel-Azeem 2015). These included soil solarization, addition of fungal antagonists, and soil amendments. Solarization alone or in combination with these antagonists and amendments reduced disease incidence. Nonetheless, *Macrophomina* continues to cause considerable losses to sesame crops in many areas.

Sesame is not at present an important commercial crop in Israel. However, we have had some experience in working with *Macrophomina* in this crop under experimental conditions in an attempt to find a reliable method of distinguishing susceptible from resistant sesame germplasm (see below).

Cotton. Charcoal rot, also known as ashy stem, is widely prevalent in cotton, *Gossypium* spp. L., growing in hot, arid areas (Abd-Elsalam 2010). Cotton cultivation is a 6-month procedure, with seeding in Israel and other semiarid countries in the early spring, when the soil is still moist from winter rains, and harvest taking place during the fall. Irrigation is applied over the course of the summer from the onset of flowering. The pathogen often obtains entry into the roots through cracks in the phelloderm; this injury is caused by the expansion and cracking of heavy soils during hot, dry weather (Sarejanni and Cortzas 1936). Initial disease symptoms, which usually begin to appear in early summer, include leaf chlorosis most likely due to toxins produced by the pathogen as it colonizes the roots, the toxins being translocated to the above-ground organs of the plants. Thereafter, the plant can exhibit dehydration symptoms despite irrigation (Fig. 1D). Another typical symptom is browning of the vascular bundles, caused by the interaction of the toxins and a plant-response product, probably gossypol. Gossypol is one of the most important sesquiterpene phytoalexins in cotton and plays a crucial role in defense against invading pathogens (Gao et al. 2013; Li et al. 2019; Wang et al. 2009) (Fig. 2).

Various methods of alleviating the attack of *Macrophomina* in cotton fields by biological control with antagonists, chemical control, quantity and timing of irrigation, and remote sensing have been proposed.

Biological control of *Macrophomina* with the fungus *Trichoderma* is used in some areas. *Trichoderma* is a widely adapted and common soil-borne fungus that is antagonistic to *Macrophomina* and alleviates disease symptoms in cotton crops (Aly et al. 2007). *Trichoderma* was effective in alleviating disease in seedlings, young plants, and mature plants, but the interaction between these two fungi varies with the isolates involved.

Chemical control of *Macrophomina* in cotton has limited efficacy. In Israel, cotton growers tend to apply fungicides as seed treatments and via the irrigation system throughout the growing season. We evaluated chemical control efficacy to optimize plant response to *M. phaseolina* (Cohen and Elkabetz 2021). In our field experiments, seed coating with azoxystrobin and Signum (boscalid, 26.7% + pyraclostrobin, 6.7%) was effective in preventing pathogen penetration of

Fig. 2. A, Symptoms of browning of the vascular bundles in cotton affected by *Macrophomina phaseolina*. B, LCMS (liquid chromatography-mass spectrometry) detection of extracts from infected bundles of cotton plants. Three chemicals were detected and identified, two toxins connected to *Macrophomina* activity, and a gossypol derivative connected to a plant defense mechanism against pathogen invasion.

seedlings for only 12 days; thereafter, the roots spread beyond the area protected by the fungicide, thus exposing them to pathogen penetration. In another experiment, three fungicide applications were made, and disease progress was evaluated 1, 2, and 3 months after seeding.

Fig. 3. *Macrophomina phaseolina* wilt incidence in cotton after three soil applications of fungicides, 30, 60, and 90 days after sowing. The fungicides were applied through the drip irrigation system, with two irrigation pipes per bed (Signum = boscalid 26.7% + pyraclostrobin 6.7%).

Azoxystrobin reduced disease incidence to 15% as compared with approximately 30% in the nontreated control and the treatments with prochloraz and prothioconazole (Fig. 3).

Timing and quantity of irrigation are key factors in cotton plant development and can affect yield and fiber quality (Dağdelen et al. 2009). Irrigation can also impact incidence and severity of *Macrophomina* in cotton fields (Cohen and Elkabetz 2021). Decades ago, irrigation technology gradually shifted from sprinklers to drip irrigation. The effect of this change on disease development is difficult to estimate due to the concurrent change of cultivars. As the common irrigation protocol is to place the irrigation pipe in the center of the bed, between the rows (Fig. 4), the water is supplied to only one side of the root system of the plants. The reason for using this procedure is to minimize the investment in irrigation equipment, but such a scheme can reduce the size of the root system and expose some of the roots to elevated soil temperatures and drought stress. Indeed, in our experiments, the roots tended to grow toward the moist soil (Cohen and Elkabetz 2021), and soil temperature was 27 and 32°C in the moist and dry sections, respectively. The parched areas of the soil were cracked, potentially causing damage to the roots and promoting *M. phaseolina* penetration. Use of either one or two irrigation pipes with or without fungicide application through the drip irrigation system was evaluated in the field. The two-pipe irrigation scheme reduced disease severity by 40%, regardless of the fungicide

Fig. 4. Differences in placement of the drip irrigation lines in cotton and the effect of irrigation on disease incidence caused by *Macrophomina phaseolina*. **A**, Diagrammatic representation of the common method of placing one irrigation line near the center of the bed, between the two rows. **B**, Diagrammatic representation of placement of two irrigation lines near the outer parts of the bed, next to each of the two rows. The blue circles indicate the diffusion of the irrigation water. The red circles indicate the diffusion of fungicide when applied through the irrigation system. **C**, Diseased cotton plants, summer 2017, when the field was irrigated with one irrigation line per bed. **D**, Healthy field, summer 2018, following careful irrigation and strict aphid control conducted to reduce plant stress. Photographs of **C** and **D** were taken at Qevuzat Yavne, central Israel, by Noam Amir.

treatment, most likely due to reducing drought stress, soil temperature, and root damage (Cohen and Elkabetz 2021). However, using two pipes per bed may be too expensive for cotton cultivation. Moreover, cotton irrigation must be carefully planned to avoid stressful situations and at the same time regulate the correct balance between vegetative and reproductive growth of the plants, to obtain optimal yields and plant height for mechanical harvesting. During the summer of 2018, a leading cotton grower demonstrated that carefully planned and conducted irrigation and proper insect management to minimize stress prevented damage from *Macrophomina* and resulted in higher yields and reduced disease incidence as compared with previous years (Fig. 4D).

Monitoring disease progress in the field is crucial for timely decision-making by growers regarding application of disease-management measures. Assessing disease location, diffusion, and severity in fields of large-scale agronomic crops such as corn, sesame, sunflower, and cotton is difficult using only conventional technologies. Applications of remote sensing technologies can provide real-time, accurate estimations of disease location and progress in large fields throughout the growing season (Zhang et al. 2019). Various sensors, such as red-green-blue (RGB), multispectral, hyperspectral, and thermal cameras can be mounted on unmanned aerial vehicles (UAVs) and other aerial platforms, and timely data can be collected from large areas in a nondestructive manner. Using such a technology, preliminary experiments were conducted in cotton fields for detection of disease development during the season. The results showed that diseased plants could be detected with both RGB and thermal cameras (Cohen and Elkabetz 2021; Sapir et al. 2018) (Fig. 5). In addition, new cotton-harvesting machinery can be equipped with a GPS, enabling yield mapping by collection of georeferenced yield data (Wang et al. 2019). Combinations of these tools can provide powerful datasets for quick, improved monitoring of disease incidence and corresponding damage to field crops in real time, facilitating focused management of the disease in the field.

Selecting Germplasm and Breeding for Resistance to Charcoal Rot

Plant breeding efforts are focused on two major clusters of traits. One of these is based on consumer expectations for quality in food crops, focusing on appearance, aroma, and flavor. The other is grower-related traits, which include all attributes that ensure efficient crop production in the target environment, including high yield, uniformity, and tolerance to abiotic and biotic stresses. For growers, disease resistance is often a trait of top priority. However, breeding for resistance to *M. phaseolina* has proven to be quite a challenge. The difficulties derive from the complex modes of inheritance of host-plant resistance to the pathogen and from the increased disease severity resulting from drier and hotter growing conditions. Accordingly, heritability of host response to this pathogen is moderate in most crops. Using sorghum (*Sorghum bicolor*), Bramel-Cox and Clafflin (1989) reported significant differences among half-sib families and among years, as well as a significant specific interaction among families and years. In bean (*Phaseolus vulgaris*), though, a relatively simple two-gene model was sufficient to explain the inheritance of resistance (Olaya et al. 1996). For cowpea (*Vigna unguiculata*), the

mode of inheritance was more complex, with nine quantitative trait loci (QTLs), accounting for 6.1 to 40.0% of the phenotypic variance, mapped using recombinant inbred line populations (RILs) (Muchero et al. 2011). Through analysis of synteny with soybean (*Glycine max*) and barrel clover (*Medicago truncatula*), candidate resistance genes were proposed within mapped QTL intervals. For soybean, a genome-wide association study (GWAS) was performed to characterize the variation and genetic architecture of resistance to *M. phaseolina* whereby 459 diverse plant introductions from the USDA Soybean Germplasm Core Collection were phenotyped in field and greenhouse screening (Coser et al. 2017). Multiple SNPs were identified but there was no overlap between the field and greenhouse QTLs, suggesting a complex molecular mechanism underlying resistance in soybean with varied responses to different environments. Overall, these results highlight the complexity involved in breeding for crop resistance to charcoal rot.

For complex polygenic traits that show strong interactions with environmental conditions, such as resistance to *Macrophomina*, backcrossing resistance into horticulturally or agronomically elite germplasm is very challenging. In horticultural crops, an alternative approach would be introgressing the resistance into rootstock germplasm (Cáceres et al. 2017; Cohen et al. 2014). By separating breeding of scions and rootstocks, much of the difficulty encountered during introgression of resistance into elite germplasm is avoided.

Successful inoculation and reproduction of disease symptoms is critical for successful completion of Koch's postulates and selecting resistant germplasm for breeding. However, achieving disease symptoms is dependent on various factors such as the appropriate inoculation method, plant age, and environmental conditions, thus there is a need to develop particular methodologies for several different hosts (Table 1). The toothpick-inoculation method (Fig. 6), for example, proved to be quite useful for melons but was not effective for watermelons (Cohen et al. 2016) and was too destructive for strawberries (Pickel et al. 2020). Furthermore, even after developing a reliable inoculation assay, there is still a need to determine whether results achieved under controlled conditions are reproducible in plants cultivated in the field.

As plant response in the field is highly dependent on inoculum density, soil type, plant age, and environmental conditions, correlating disease development under field conditions with results of experiments conducted under greenhouse or controlled conditions is a key requirement that must be addressed for each tested host. Another obstacle in correlating greenhouse results with performance in the field is that plants are inoculated in the greenhouse with an artificially produced isolate, but in the field, there is no assurance that the plants will react to the pathogen in a similar manner. Additional organisms in the rhizosphere of the field, acting in concert with the pathogen, can greatly influence disease reaction (Martyn 2008).

We screened melon, strawberry, sesame, and cotton in Israel in an attempt to identify germplasm having resistance to *Macrophomina*. Below, we summarize our results and compare them with those obtained by researchers in other countries, and describe the challenges we encountered in the initial phases of breeding for resistance in these crops.

Melon. For melons, the toothpick-inoculation method, whereby microsclerotia are produced aseptically on the wooden toothpicks that are subsequently used to inoculate the basipetal part of the stem and crown tissues of tested plants, has proven to be quite reliable and useful (Cohen et al. 2016) (Fig. 6). This method reproducibly creates typical charcoal rot symptoms, and the symptoms that develop are similar to those seen in natural infections. It has been adopted in screening melon accessions for resistance to *Macrophomina* (Fig. 7) and in screening assays of disease control measures, such as fungicide application.

Using this method, Ambrósio et al. (2015) evaluated melon seedling resistance to charcoal rot across 97 *C. melo* accessions and observed that some accessions developed milder symptoms than others. de Sousa Linhares et al. (2020) tested the effect of various temperatures on the response of six melon accessions to infection and observed that charcoal rot severity increased with exposure of

Fig. 5. Aerial views of cotton field. A, Yellow focal point indicating initial *Macrophomina phaseolina* symptoms. B, image taken by a thermal camera.

plants to elevated temperatures. Although elevated temperatures caused an increase in severity of disease symptoms in most genotypes, this effect was less pronounced in the accessions that were most susceptible and those that were most resistant. Thus, there is an important genotype-by-environment interaction for severity of disease expression.

Recently, we evaluated 25 melon accessions under greenhouse and open-field conditions with the goal of identifying a dependable screening protocol to assist in breeding for tolerance to *Macrophomina* (Cohen et al., in preparation). We, too, observed genetic variation among melon accessions in response to *M. phaseolina* (Fig. 8) and strong genotype-by-environment interaction in accessions having an intermediate response. We also tested the response of 15 F₁s, derived from half-diallels of six selected tolerant and susceptible parents, to *M. phaseolina* and observed a significant correlation between parental means and hybrid response. Overall, the hybrids were more tolerant than their parents. These results suggest that tolerance of melons to *M. phaseolina* has both additive and dominant components. However, to date, no QTLs or trait-linked markers for tolerance to this pathogen have been reported for melons.

Trait heritability is a fundamental property in defining the efficiency of breeding and selection. It is clear that the response of melon to *M. phaseolina* infection is affected by environmental conditions, and that the heritability of this trait is moderate and, similar to other complex traits, requires focused breeding for target environments. The fact that accessions on the tails of the distribution are consistent across environments in their susceptible or tolerant phenotype is encouraging, as these accessions most likely reflect genetic combinations that are fixed for the relevant QTLs.

Strawberry. *Macrophomina* is the major soil-borne disease affecting the nursery and production stages of strawberry crops in Israel and other countries having warm climates, spurring research and breeding of strawberry for resistance to this pathogen. ‘Seascape’ grown in California (Koike 2008) and ‘Albion’ and ‘Aromas’ grown in Western Australia (Fang et al. 2012) were among the first strawberry cultivars identified as showing tolerance to *Macrophomina*. Recently, more strawberry cultivars have been reported with relative tolerance to this pathogen, including ‘Coral’ in Spain (Avilés et al. 2012), ‘Strawberry Festival’ in Chile (Sánchez et al. 2016), and

‘Osceola,’ ‘Manresa,’ and ‘Megan’ in California (Holmes et al. 2017). In trials conducted in Israel, ‘Pelican’ from the southeastern U.S.A. (Smith et al. 1998) was relatively tolerant, whereas ‘Albion’ was one of the most susceptible cultivars (Pickel et al. 2020). In summary, tolerance to *Macrophomina* in strawberry appears to be dependent on particular combinations of cultivar and growing region. To our knowledge, no source of high resistance has as yet been reported, but two QTLs have been associated with partial resistance (Nelson 2018).

Developing a reliable inoculation technique for *Macrophomina* charcoal rot in strawberry is imperative for efficient screening of germplasm for resistance. The toothpick-inoculation method was found to be too aggressive and not reliable for screening in strawberry, as all plants regardless of cultivar collapsed and died within 1 to 2 weeks (Pickel et al. 2020) (Fig. 9A). However, a reliable technique was developed based on artificial inoculation of growth medium with artificially produced sclerotia (2.5×10^3 sclerotia/g soil) in a soil mix (Fig. 9A). Accordingly, 33 strawberry accessions were grown under natural outdoor conditions in a screen house and compared. An array of reactions, from highly susceptible to tolerant, was observed among the tested accessions (Fig. 9B) (Pickel et al. 2020).

Fig. 6. A, Wooden toothpicks placed on a Petri dish with *Macrophomina phaseolina*. B, Close-up, the toothpicks are colonized by hyphae that form into microsclerotia within 3 to 4 days. It is recommended to use freshly prepared toothpicks that contain viable mycelium ensuring successful inoculation.

Table 1. Summary of the efficacy of inoculation methods on symptom development and suitability for germplasm selection using the toothpick method, drench of macerated fungal culture around the crown area, and artificial inoculation of growth media (only for strawberry)

Host	Inoculation method				
	Toothpicks		Soil drench		Growth medium
	Symptom development	Suitability for germplasm selection	Symptom development	Suitability for germplasm selection	Only for strawberry
Melon	Identical to natural infection	High correlation between pots and field inoculation	Identical to natural infection	Not tested	
Watermelon	Caused wilt in the field; no disease development in pots	Not tested	Caused wilt in the field; no disease development in pots	Not tested	
Strawberry	Too aggressive	Not tested	Not tested	Not tested	Artificially produced sclerotia (2.5×10^3 sclerotia/g soil)
Sesame	Disease did not develop in pots and field from basipetal stem inoculation	Disease development from acropetal stem inoculation in pots had high correlation to natural infection in field	Ineffective in pots and field	Ineffective, widely varying results	Suitable for symptom development and germplasm screening
Cotton	Disease did not develop in field trials	Not tested	Symptoms developed only in pots, as slower plant development and root rotting	Unsuitable, results in pots did not match plant response in the field	

Although no resistant cultivars have been identified to date in Israel, the relatively tolerant 'Pelican' and 'Orly,' the latter an Israeli cultivar, are good candidates for future breeding efforts to develop resistant strawberry germplasm.

Breeding for resistance to *Macrophomina* in strawberry can be expected to be a prolonged and difficult process. An optional strategy for resistance breeding might be: (a) select a few tolerant strawberry accessions as donors; (b) combine (hybridize) these tolerant accessions with a few elite strawberry cultivars using regular hybridization methods to produce several thousand seeds; (c) germinate and propagate individual seedlings in *Macrophomina* preinoculated substrate (separately in trays), under suitable conditions for *Macrophomina* disease development; (d) further screen the most tolerant seedlings for other horticultural traits in breeding plots during the fruiting season; and (e) further evaluate the advanced selections from this breeding program for *Macrophomina* resistance.

Fig. 7. Toothpick inoculation of melon and watermelon with *Macrophomina phaseolina*. **A and B**, Outer and inner symptoms, respectively, in the stem of melon. Rotting of the soft tissue within the stem and rapid formation of black microsclerotia on the dead tissue. **C**, Watermelon, normal corky tissue at the stabbing site not related to the inoculation. **D**, Watermelon, small wound at the stabbing site similar to that in the sterile control.

Fig. 8. Inner rotting of the stem of three melon accessions following inoculation with *Macrophomina phaseolina*-infested toothpicks. Left, severe rotting in 'Piel de Sapo Redon' (PSR), rated as highly susceptible (HS). Center, moderate rotting in 'Ananas Yoqne'am' (AY), rated as susceptible (S). Right, rotting only at stabbing site in 'Qishu Meshullash' (QME), rated as resistant (R).

Sesame. In Israel, we observed natural infection of sesame by *M. phaseolina* in a field in which the previous crop was melons. Early wilting, at a phenological stage of 7 to 10 true leaves, was evident only in a few plants belonging to different accessions, but significant plant mortality occurred at flowering. Typical symptoms of wilting were observed but rotting was not evident (Fig. 1C).

There is a great need in sesame to develop a screening procedure that, applied to young plants, could reliably identify resistant germplasm. To be useful, this procedure would have to accurately predict the behavior of the tested accessions in the field. Tabassum et al. (2014) successfully reproduced disease symptoms 17 days after toothpick inoculation of sesame plants under field conditions. Dinakaran and Mohammed (2001) conducted parallel evaluation of plant response to the pathogen in field and pot experiments of six sesame accessions, using soil amended with artificially infested sorghum seeds. They reported similar results in pots and the field. Nonetheless, it is important to take into consideration that *M. phaseolina* has a high level of genetic polymorphism based mainly on geographic origin (Babu et al. 2010; Mahdizadeh et al. 2012), so the scope of applicability of these results may be limited.

Fig. 9. **A**, Strawberry plant mortality (percent dead plants) over time (days after inoculation) using two different inoculation methods, toothpick (full symbols) and growth medium with artificially produced sclerotia at 2.5×10^9 microsclerotia/g soil (empty symbols), of two accessions, the tolerant 'Hadar-359' and the susceptible 'Festival-730.' Each data point represents the mean of 16 replicate plants. Vertical bars represent the least significant difference at $P < 0.05$ as determined by Tukey's honestly significant difference. **B**, Symptoms of *Macrophomina phaseolina* and selection of strawberry accessions under greenhouse conditions.

We inoculated the basipetal part of the stem of young sesame plants of various accessions in the greenhouse, using *Macrophomina*-infested toothpicks, and obtained a differential response. Purple lesions developed in some accessions, whereas in others, no symptoms or color changes were observed (Fig. 10A and B). However, some plants of both groups exhibited disease symptoms long after inoculation. Therefore, it was not possible to rely on a differential response to inoculation at an early stage of plant development as a dependable method for selection.

The same accessions of sesame that were tested in the greenhouse were then inoculated in the field, 3 weeks after emergence. Again, inoculation of the lower stem showed penetration of the pathogen into the plant tissues but little or no disease developed. The point of inoculation of the lower stem responded to the inoculation by swelling, which is probably a resistance mechanism (Fig. 10C and D). A cut made in the lower stem revealed some rotting of the soft parenchyma tissues within, which did not spread to the dense tissue surrounding the vascular bundles.

We then modified our greenhouse methodology by inserting the infested toothpicks into the soft tissue of the acropetal part of the stem (Fig. 10E and F). This resulted in clear differences among the over 30 tested accessions in lesion size, and in some of them, lesions did not develop at all. When eight of these accessions were then grown out in

the field, we observed a fairly high correlation ($r = 0.67$) between disease reaction to acropetal stem inoculation in the greenhouse and disease incidence under natural field conditions. Nonetheless, it would be desirable to calibrate this modified method in an effort to achieve even more highly correlated results.

Cotton. During the 1970s, cotton cultivation based on Acala-type (*Gossypium hirsutum*) cultivars, originally imported from California, was very popular and profitable in Israel. During the past 20 years, as a result of global economic changes, cotton cultivation in Israel decreased dramatically and shifted to extra long staple (ELS) Pima-type (*G. barbadense*) cultivars that generated higher economic value for cotton growers. Disease severity appears to be greater in the present-day fields of Israeli Pima-type cultivars.

M. phaseolina can cause disease following artificial inoculation of young plants in the greenhouse and mature cotton plants in the field. However, it seems that the plant can coexist with the pathogen asymptotically. Colonization of the plants leads to symptom appearance mostly when environmental conditions such as elevated temperatures (air and soil) or drought stress occur.

The relationship between inoculum level, root colonization, disease severity, and their relevance to cotton cultivation were evaluated (Cohen and Elkabetz 2021). Young cotton plants were inoculated in the greenhouse by drenching the soil with *M. phaseolina*. However, the disease level observed in the various evaluated cotton lines did not correspond with the knowledge or the expectations of the breeders as to the response of the plants in the field. Therefore, the approach used by the cotton breeders in Israel is to conduct the selection for disease resistance and high yields in naturally infested fields, while exposing the plants to moderate water stress to improve selection of the best performing accessions.

The interspecific cotton hybrid 'Acalpi,' obtained in crossing Acala- with Pima-type germplasm, was developed recently by Hazera Seed Company in Israel. The purpose was to combine the yield attributes contributed by the Acala-type cottons with that of quality contributed by the Pima-type cottons (Basbag and Gencer 2007). The new hybrid is less susceptible to *M. phaseolina*, probably as a result of heterosis causing vigorous vegetative growth and better adaptation to drought stress.

Conclusion

The interaction between *M. phaseolina* and various crop hosts differs from almost every perspective. Pathogenicity expressed as wilting accompanied by rotting results from the activity of degradation enzymes, whereas dehydration symptoms appear to be mainly the result of toxin activity. For melon and watermelon crops in Israel, disease can be managed effectively using both fungicides and grafting. For strawberry, preplant treatments with fumigants helped alleviate losses and later planting delayed the onset of the disease. For cotton, fungicidal control has not had adequate effectiveness in Israel, but appropriate, timely irrigation seems to be important for reducing losses to *Macrophomina*. Breeding for resistance is the preferred approach for disease management. However, the moderate to low degree of heritability of resistance and the relatively large effect of the environment on disease incidence and severity confounds breeding for resistance. Specialized techniques and methodologies need to be developed for each crop and region individually to identify and facilitate enhancement of resistant germplasm. For example, the toothpick-inoculation method applied to young melon plants in the greenhouse creates symptoms identical to those observed in natural field infections, but it is too aggressive for strawberry and ineffective for plants with hard, compact stems, such as watermelon, sesame, and cotton. However, a modification of this method has provided promising results with sesame. Given the wide-ranging effects of the environment on the development of charcoal rot, the results we have described here may be less applicable to other parts of the world in which a very different climate prevails. A combination of classical breeding, based on direct phenotypic selections, with genotypic selections using QTL-linked markers or genomic markers, is a potential future strategy for development of elite germplasm resistant to

Fig. 10. Toothpick inoculation of sesame plants. **A** and **B** show inoculation at the stem base. **A**, Development of purple lesion at the infection site. **B**, Stab wound at the infection site. Disease did not develop in both cases. **C** and **D** show stem-based inoculation in the field. **C**, Rotting of the soft parenchyma tissue in the interior of the stem while the outer tissues comprising the vascular systems were not affected. **D**, Swelling of the stem tissue at the infection site; the plants did not show disease symptoms. **E** and **F** show inoculation at an acropetal portion of the stem. **E**, Lesion development. **F**, Symptomless stem.

M. phaseolina. As global warming continues, the environment in many regions will become more favorable to *M. phaseolina*, which can be expected to induce more severe outbreaks of disease and expand its natural range. Hence, there is some urgency to identify and enhance sources of resistance to this pathogen in the most susceptible agronomic and horticultural crops.

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Harry S. Paris is a senior scientist in the Cucurbits Section, now retired and continuing as a volunteer, of the Agricultural Research Organization at its northern campus, the Newe Ya'ar Research Center. He received his B.Sc. in biology from the State University of New York College at New Paltz in 1973 and his Ph.D. in horticulture from Rutgers – The State University of New Jersey in 1978. He joined the A.R.O. in 1978 and retired in 2018. Dr. Paris has devoted his entire professional career of 40+ years to research and breeding of cucurbits. He has authored over 100 peer-reviewed articles on cucurbits and authored or edited over 30 books and book chapters. Several squash cultivars that he bred have achieved national or international recognition. He was bestowed the A.R.O.'s Lifetime Achievement Award in 2016.

Amit Gur

Amit Gur is a research scientist in the Cucurbits Section of the Agricultural Research Organization at its northern campus, the Neve Ya'ar Research Center. He received his Ph.D. in genetics and plant breeding in 2004 from the Hebrew University of Jerusalem (Israel), where he worked with Prof. Dani Zamir on tomato genetics. He then occupied a post-doctoral position, from 2005 to 2007, at Cornell University, where he studied corn genetics. From 2008 to 2015, Dr. Gur worked as a geneticist at the Monsanto Vegetable Division in Israel and, in 2015, he joined the A.R.O. His research focuses on the genetics of yield and fruit quality of melon, watermelon, and squash.

Nir Dai

Nir Dai is a senior scientist in the Department of Vegetable Sciences of the Agricultural Research Organization at its central campus, the Volcani Center. He received his Ph.D. from the Hebrew University of Jerusalem, where he specialized in the biochemistry of fruit sugar metabolism. Dr. Dai joined the A.R.O. in 2004 and has served as leader of the strawberry breeding program there since 2006. The newly released cultivars today comprise 80% of the Israeli strawberry market. Other research interests include flower induction, factors affecting yield and fruit quality, and pests and diseases of strawberry. Over the past six years, commercial cultivation of raspberries and blueberries, new to Israel, are part of his research efforts. For raspberries, the primary aim is to select for heat tolerance. For blueberries, the primary aim is adaptation and designing protocols for cultivation in various parts of the country.

Onn Rabinovitz

Onn Rabinovitz is an extension agent of field and vegetable crops with Migal and with the Northern Region Research and Development, at Qiryat Shemona, Israel. He received his Ph.D. in 2006 from the Hebrew University of Jerusalem, Israel. Until 2018, he was an extension agent with the Agricultural Extension Service, Field and Vegetable Crops Unit, of Israel's Ministry of Agriculture.

Stanley Freeman

Stanley Freeman is a senior scientist in the Department of Plant Pathology and Weed Research of the Agricultural Research Organization at its central campus, the Volcani Center. He received his Ph.D. in plant pathology from the Hebrew University of Jerusalem in 1991 and was awarded a two-year BARD post-doctoral fellowship to conduct research at the University of California, Riverside. He joined the A.R.O. in 1993, where he has worked primarily on fruit-crop diseases. His expertise encompasses the fields of molecular plant-fungal-pathogen interactions, including *Macrophomina*, *Fusarium*, and *Colletotrichum*. Dr. Freeman has authored over 100 peer-reviewed articles and 15 book chapters, and he has edited two books. He has supervised over 30 M.Sc. and Ph.D. students as well as post-doctoral fellows from universities in Israel and abroad. He formerly served as the president of the Israel Phytopathological Society and currently is the co-editor-in-chief of the peer-reviewed journal *Phytoparasitica*.