

Research Article

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Challenges With Use of Grafting for Mitigation of Soilborne Disease in Chile Pepper Production

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Abstract

Chile pepper cultivation faces significant challenges including viral, bacterial, and fungal pathogens, leading to substantial yield losses worldwide. Conventional disease management strategies often rely heavily on agrochemicals, raising the concern of environmental and health risks. Grafting is an option for mitigating many diseases while promoting sustainable agricultural practices. Grafting is especially attractive when natural genetic resistance is absent in elite cultivars but present in lines that have poor agronomic characteristics. *Phytophthora capsici* is the most damaging pathogen in chile production across the southwestern US, and currently there is no useful genetic resistance in any elite cultivars used in commercial chile production. Robust *P. capsici* resistance is however present in several chile lines, like CM-334, that are not used in commercial production due to poor agronomic characteristics including small fruit size, poor plant habit, and low yield. The efficacy of grafting as a sustainable strategy to mitigate *P. capsici* infection in chile has not been widely explored. Here we present results from research examining the possibility of using CM-334 chile as a resistant rootstock to create grafted *P. capsici* chile plants. The results of this work show that several elite chile cultivars are compatible with, and could be successfully grafted onto, CM-334 rootstock to create *P. capsici* resistant plants. Unfortunately, these plants are unlikely to be commercially useful due to the propensity of CM-334 to generate large numbers of adventitious shoots that result in plant canopies with mixed CM-334 and elite cultivar branches. Unless these adventitious can be suppressed, the resulting plants will not be useful for commercial production due to a very bushy architecture and mixed CM-334 and elite cultivar fruit that would be difficult to separate. The bushy plant habit would also make fruit difficult or impossible to harvest mechanically. Further, the mixed flowers in the canopy would negate the use of these grafted plants for the production of open pollinated seeds since the high number of CM-334 flowers in the canopy would make it virtually impossible to avoid cross pollination. This work shows that while grafting can be used to create plants resistant to *P. capsici*, and likely other soilborne diseases, it is unlikely to be commercially viable until disease resistant rootstock lines with better agronomic characteristics are identified.

Introduction

Grafting is a well-established method in plant disease management that has been employed for improving crop plants for thousands of years [1]. In modern agriculture grafting is practiced in many annual and perennial crops including melons, squash, cucumber, solanaceous crops like tomato and pepper, fruit trees,

ornamentals, and more. Grafting has been used to confer a variety of benefits to vegetable crops including improved plant vigor [1] and nutrient utilization efficiency [2], improved abiotic stress tolerance [3,4], and improved tolerance to soilborne diseases [5-10]. Grafting can also improve economic returns to growers and sustainability

in production due to reduction in pesticide use [11]. And since grafting utilizes plant genetics rather than synthetic chemistry, grafting to improve resistance to biotic and abiotic stress is fully compatible with organic agriculture [11].

The widespread adoption of grafting in commercial agriculture reflects its economic and environmental benefits. One major advantage is the reduction of chemical inputs. By selecting disease-resistant rootstocks, farmers can reduce or eliminate the need for fungicides, nematicides, and other pesticides. This is particularly important in organic farming, where chemical options for disease control are limited. In conventional farming, grafting allows for more targeted and limited use of chemical treatments, thus reducing environmental pollution and lowering production costs [11].

Grafting is a common practice for tomato production throughout the world that has been used for control of various diseases [12] as well as to improve tolerance to abiotic stresses like temperature stress [4,13] and salt stress [14]. Grafting has been specifically used to reduce losses to diseases like bacterial wilt caused by *Ralstonia solanacearum* and Fusarium wilt caused by *Fusarium oxysporum f.sp. lycopersici* [11]. While grafting is commonly used in tomato production, its use in pepper production is limited. Lee, et al. [15] performed a broad survey of grafting in vegetable production. Data available at that time indicated that grafting is used only in commercial production sweet green peppers, and that grafting was only used to generate about 5% of the peppers used in commercial production, with all of this occurring in Asia and none in the US.

Chile pepper (*Capsicum annuum* L.) is highly susceptible to a number of soilborne fungal diseases. *Phytophthora capsici*, *Verticillium dahliae*, *Fusarium oxysporum*, *Rhizoctonia solani* which combine cause substantial losses on chile pepper worldwide. *P. capsici* and *V. dahliae* are the most prevalent and severe diseases of chile in the Southwestern US [16]. There is no useful genetic resistance to any of these pathogens in elite cultivars used for pepper production, and chemical control options are limited or ineffective. Development of grafting for control of these diseases would greatly benefit chile pepper producers, especially the growing number who are transitioning to organic production.

Resistance to some of these pathogens is present in *Capsicum* landraces breeding lines derived from crosses with landraces and legacy stock. While none of these perform well enough to be used for commercial chile pepper production, they may be suitable for use as rootstock to support scions from current elite commercial lines. The use of cultivars "Graffito" and "Gc 1002" as rootstock produced *P. capsici* tolerant grafted chile plants [17]. Grafted plants having "Graffito" rootstocks also showed improved tolerance to root knot nematode (*Meloidogyne incognita*), a significant pest on chile peppers worldwide. This prior work shows the potential for grafting to address pathogen and pest problems in chile pepper production.

The chile line CM-334 is a land race of chile from Mexico that is known to have high levels of resistance to several major pests and pathogens of chile including *P. capsici* [18] and several species of root knot nematode [19]. Here we describe experiments evaluating

the potential of CM-334 as a rootstock to produce grafted plants containing scions from "Machete", a current elite cultivar of chile widely used in commercial production in the southwestern US and Mexico. The goal of this work was to determine if CM-334 were compatible and could be successfully grafted and, if so, whether the resulting plants would be useful for commercial chile production. Our results show that CM-334 and Machete could be grafted and that the resulting plant thrived while being resistant to soilborne *P. capsici*. However, the growth habit of CM-334 renders the plants unattractive for commercial use. Specifically, the highly branched plant architecture of CM-334 resulted in grafted plants that had many CM-334 branches in the canopy, which would result in mixed fruit. These plants would also be unusable for production of open pollinated seeds since many of the CM-334 branches flowered and could potentially pollinate flowers in the elite cultivar scion.

Methods

Seed for chile pepper (*Capsicum annuum* L.) lines CM-334 and Machete were obtained from Curry Farms (Pearce, AZ). Seeds were planted in 36 cell flats or small Styrofoam cups filled with potting mix (Miracle-Gro moisture control potting mix, <https://miraclegro.com>). Seedlings for both lines were grown until the first or second set of true leaves emerged. Figure 2 shows various steps of the grafting process. At this point they were grafted by making 45° cuts in both the rootstock and scion. Cuts were made in the same position on both the rootstock and scion, either just below or just above the cotyledon leaves. Rootstock and scion portions were joined using appropriately sized silicone grafting clips, usually 1.5, 2.0, or 2.2mm (Johnny's seeds, <https://www.johnnyseeds.com/>). Grafted plants were placed under humidity trapping domes and covered with a black garbage bag to keep them dark for 72-96 hours. A fine mist sprayer was used to water plants and keep foliage moist during the first 96 hours after grafting. After 96 hours the humidity was slowly reduced by opening the vents in the covers. Covers were removed after plants were acclimated to normal humidity, usually 3-4 days after opening vent covers fully.

P. capsici inoculation assays were performed as previously described [20]. Briefly, zoospores were prepared from *P. capsici* strain PWB-9 and counted using a hemocytometer. Young plants (6-12 leaf stage) were inoculated with 10,000 zoospores per plant in 10 ml of water delivered through a disposable sterile 10 ml pipet. Plants were watered to soil saturation at the time of inoculum to mimic flooding in an agricultural field and watered normally thereafter. Plants were monitored for disease symptoms starting 4 days after inoculation [21-25].

Results and Discussion

The chile line CM-334, which is known to be resistant to several soil borne fungal pathogen and root knot nematode was chosen for use as rootstock in these experiments. We verified the *P. capsici* susceptibility of both plant lines prior to starting this work. Several CM-334 and Machete plants were inoculated with *P. capsici* using 10,000 zoospores per plant. Figure 1 shows flats of CM-334 and Machete at the time of inoculation (panels A and B) and 14 days

after inoculation (panels C and D). As expected, the Machete plants started showing symptoms within 7 days of inoculation with all inoculated plants having died by 14 days after inoculation (Figure 1D). In contrast, similarly aged CM-334 plants inoculated at the same time with the same zoospore preparation perfectly showed

no symptoms at day 14 (Figure 1C) and remained perfectly healthy for the duration of the experiment (not shown). This showed that the lines chosen to test if grafting could be used to protect Machete scions from *P. capsici* behaved as expected.

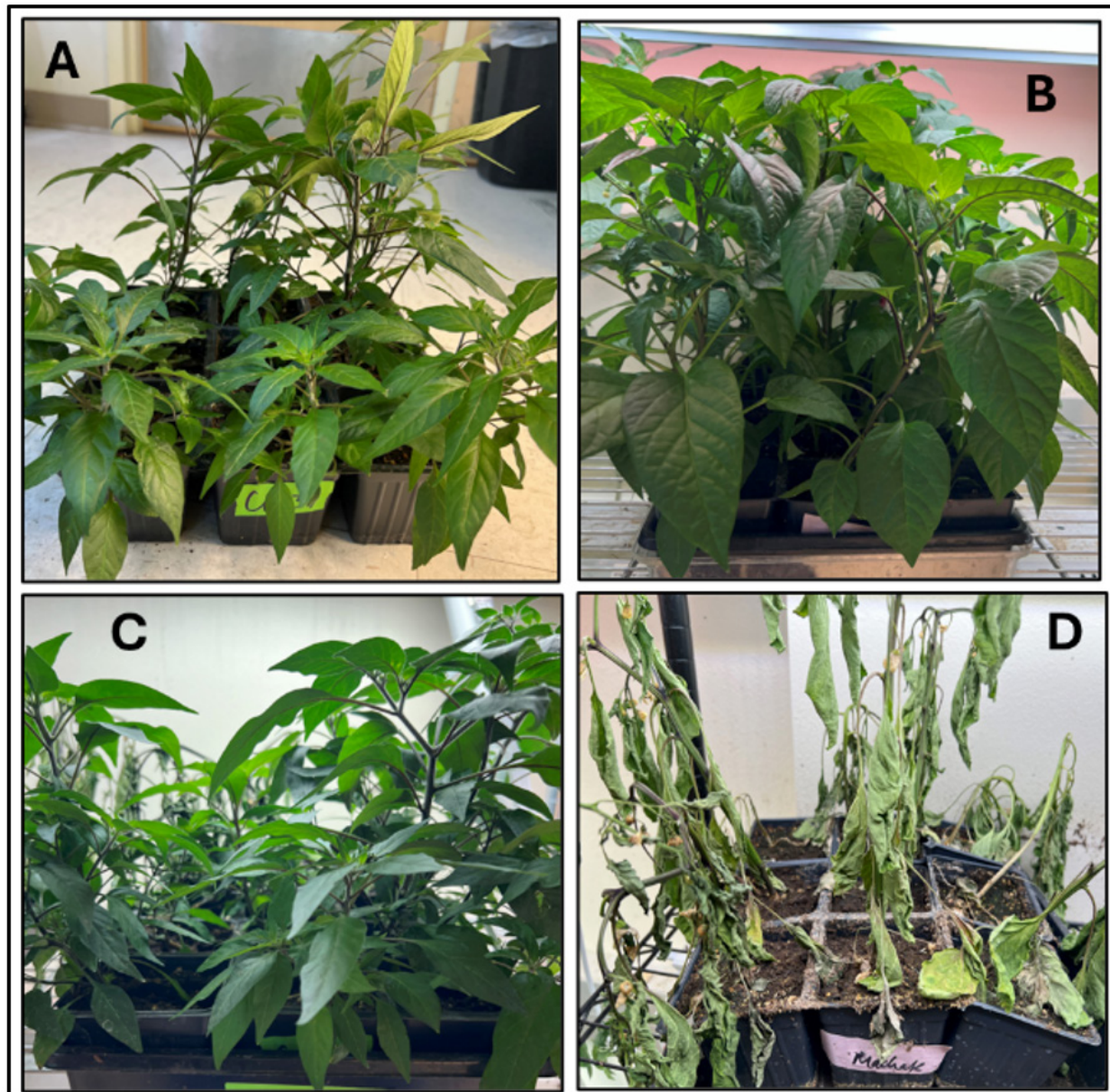


Figure 1: *Phytophthora capsici* susceptibility test.

Chile lines CM-334 (panels A and C) or Machete (panels B and D) were inoculated with 10,000 *P. capsici* zoospores as described in the methods section. Panels A and B show plants at the time of inoculation, while panels C and D show plants are 14 days after inoculation.

Scions from the elite commercial line Machete were grafted onto CM-334 rootstock as described in the methods section. Figure

2 shows the various steps in the process from starting plants through grafting and a fully healed graft junction.

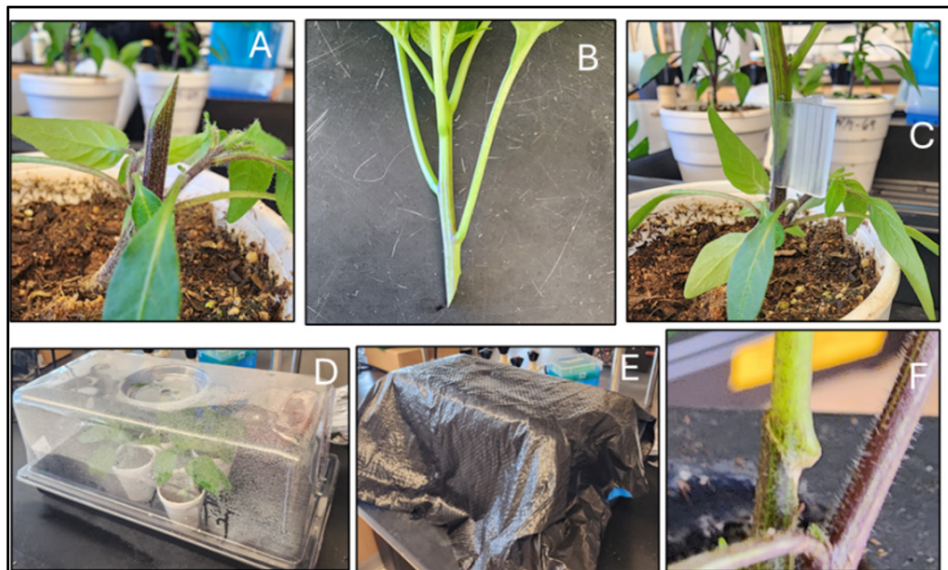


Figure 2: Stages of grafting.

CM-334 rootstocks and Machete scions were cut at a 45° angle (panels A and B respectively) then using a silicone grafting clip (panel C). Grafted plants were placed under humidity domes (panel D) and covered with black plastic to keep them dark (panel E). A healed graft junction with the grafting clip removed is shown in panel F.

We found that scions from the elite commercial line “Machete” were compatible with the CM-334 rootstock with successfully grafted plants surviving and growing well with no signs of incompatibility at the grafting site. Unfortunately, as shown in Figure 3 the CM-334 rootstock produced numerous adventitious

shoots that grew into the Machete canopy. This happened with every plant and resulted in grafted plants having canopies that contained ~30-50% CM-334 branches. While these plants were resistant to *P. capsici* (not shown) they are not useful for commercial production for several reasons.



Figure 3: Adventitious shoot from CM-334 rootstock (circled in red) growing up and invading the canopy formed by the Machete Scion.

One problem is the difference in fruit types produced by CM-334 and elite cultivars like Machete. The fruits produced by CM-334 are much smaller and hotter (spicier) than fruits produced by elite cultivars like Machete (Figure 4). Grafted plants with mixed canopies will have lower yields due to the smaller fruit on the CM-

334 branches and be more difficult to harvest due to the mixed fruit types. In addition, it will be impossible to use these plants to increase seed since the presence of CM-334 flowers in the canopy is likely to contaminate the Machete flowers via cross pollination.



Figure 4: Comparison of fruits.

Fruit from the elite production chile line Machete (top) are much larger than those from CM-334 (bottom). In addition to the size difference, Machete puts on many more fruit per plant than CM-334. And while the Machete fruits have low heat levels and a pleasant chile flavor, the CM-334 fruit and much hotter than typical commercial chile lines.

The results of this work demonstrate that grafting is an approach that can be used to reduce losses to soilborne disease like *P. capsici* in chile production. However, for this to be commercially viable chile rootstock lines that are disease resistant but do not produce adventitious shoots that invade the scion canopy will need to be identified or developed.

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Conflict of Interest

No conflict of interest.

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