# Sources of Inoculum, Biology, Epidemiology, and Transmission of Sour Rot of Stone Fruit and Management of the Disease in the Orchard

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**INTRODUCTION**

In early July 2001, various samples of nectarine and peach fruit from orchards in northern Tulare and in Fresno counties and from packinghouses in this area were brought to our laboratory for diagnosis of an unusual decay. When the decay lesions originated close to the stylar end, leaking juice streamed from the lesions. When the lesion was on the stem end of the fruit and touched the packing box, it developed a ring-shape decay of 0.5 to 2.0 cm inner and 1.0 to 3.0 cm outer diameter. The leaking juice dissolved the cuticle, the epidermis, and the outer layers of the flesh, creating distinct furrows in the fruit tissue. Samples with similar decay lesions were observed and isolations were made several times during 2001-2007.

Isolations from these fruit consistently yielded *Geotrichum candidum* frequently along with two other yeasts, which were identified as *Issatchenkia scutulata* and *Kloeckera apiculata*. *G. candidum* was isolated more frequently than the other two yeasts. Pathogenicity tests were performed with all three organisms, and we concluded that each of these yeasts by itself and in combination with one or both of the others was able to cause sour rot decay on stone fruit (Michailides et al., 2004). Because *G. candidum* was shown to be more aggressive than the other yeasts and was the most frequently isolated microorganism, all the inoculation and transmission experiments were done using *G. candidum* isolates.

During 2005 to 2007 significant progress was made in understanding the biology of the main cause of sour rot (*G. candidum*) and the various factors affecting the disease in California stone fruit orchards. Specifically, the objectives in 2007 were:

## OBJECTIVES

1. Determine differences in pathogenicity and polygalacturonase production by *G. candidum* corresponding to various levels of virulence.
2. Complete the study on stone fruit cultivar susceptibility.
3. Determine whether *G. candidum* multiplies in the packinghouse.
4. Identify sanitation practices in harvest equipment and the field which can reduce sour rot decay.

## METHODS AND RESULTS

### 1. Determine differences in pathogenicity and polygalacturonase production by *G. candidum* corresponding to various levels of virulence.

In 2006, we compared 41 isolates of *Geotrichum candidum* isolated from soil, decayed fruit, packing lines, and surfaces of peach leaves and fruits for their pathogenicity and found that all of them were pathogenic with some differences in pathogenicity. In 2007, we tested *Geotrichum citri-aurantii* the causal agent of sour rot on citrus. An isolate of *G. citri-aurantii* was grown on acidified potato-dextrose agar for 72 hours, and a $10^6$ spores/ml suspension was prepared and used to inoculate nectarine fruits. The isolate was tested in a randomized complete design with four replicates and three subsamples. Nectarine fruits were surface sterilized by dipping them in a solution of 160 ml of chlorine, 160 ml ethanol, and 0.5 ml Tween 20 surfactant in 10 liters water for 4 minutes. Fruits were placed on a raised plastic mesh in plastic containers and water was added to the bottom of the containers to create high humidity (close to 100% relative humidity). Fruits were wounded and inoculated with 20 µl of $10^6$ spores/ml. The plastic containers were incubated on a laboratory bench (74°±2°F). Pathogenicity of the isolates and lesion diameter were recorded after 5 days inoculation.

*Geotrichum citri-aurantii* was found to be pathogenic on nectarine fruits and caused and average lesion size of 18.7 mm. The citrus strain is considered very pathogenic if compared to the most pathogenic isolates of *G. candidum* causing the highest decay on nectarine fruits (**Fig. 1).** This result suggests that the citrus strain may be as virulent as the isolates collected from decayed stone fruits, soil, and packing house isolates. More citrus isolates is need to confirm this result. Also this result suggests that extra caution needs to be taken when processing stone fruits and citrus fruits with the same packing line at the end of the citrus packing and the beginning of the stone fruit season, as well as to dispose cull citrus fruits away from stone fruit orchards.

*Polygalacturonase (PGase) production:* Polygalacturonase activity of 18 isolates of *G. candidum*, and one isolate of *G. citri-aurantii*, used for the pathogenicity experiments was used in this experiment. *Geotrichum* isolates were grown in 50 ml of yeast extract pectin broth (0.3% yeast extract and 0.5% pectin). The cultures were inoculated with a spore suspension of each *Geotrichum* isolate. A negative control was inoculated with sterilized water and incubated at 77°F for 72 hours in the dark on a rotary shaker operated at 100 rpm. Cultures were filtered using a 0.45-µm Millipore filter and the samples were stored at -4°F until used for analysis. A quick method, the cup-plate assay, was used to determine any differences among the *Geotrichum* isolates. Media for the cup-plate enzyme assay were prepared using sodium polypectate as a substrate for polygalacturonase and poured into plates of 15 cm in diameter. Wells were created by removing agar plugs with a #2 cork borer, and 100 µl of each sample were dispensed in each well as, and each sample was duplicated in a different well on the same plate. Possible relationships between PGase production and pathogenicity were determined. Differences among pathogenicity, virulence, and PGase activity will indicate variability in the pathogen's population. Great diversity in pathogen population suggests that control can be more difficult than with a uniform fungal population.

There were differences in enzyme production by different isolates using the cup- plate assay. Enzyme activity was as low as 9.5 mm for isolate 16 and 20 mm for isolates 1, 3, and 4 (**Fig. 2A**). To decide if disease severity represented by lesion size is dependent upon enzyme production, lesion size was plotted against enzyme production. R square explaining the relationship between the lesion size and enzyme production was low (0.11). This is due to the fact that there were few highly pathogenic isolates that produced low amounts of enzyme as compared to other isolates. In general, a trend of increased severity was associated with increased production of polygalacturonase (**Fig. 2B**). Citrus strain production of polygalacturonase was among the highest producing isolates and the activity zone was 18.5 mm. The production of polygalacturonase is an important pathogenicity factor among bacterial and fungal pathogens causing soft rot and post harvest rots by dissolving the middle lamella that adheres plant cells together, resulting in the disintegration of plant tissues. A more accurate method (i.e., **viscometry**) will be used to look more closely at the differences among different isolates which may suggest a more diverse population of *G. candidum*.

**2. Complete the study on cultivar susceptibility.**

In 2006, we compared several varieties of peaches and nectarines and we found some differences in susceptibility. In 2007 we evaluated 36 varieties for their susceptibility to sour rot. Fruits were collected from commercial orchards that have not been treated with fungicides. Fruits were surface sterilized by dipping them in a solution of 160 ml of chlorine, 160 ml ethanol, and 0.5 ml Tween 20 surfactant in 10 liters water for 4 minutes. Fruits then were placed randomly on plastic trays and placed in boxes in a randomized complete block design with 8 replicates and 4 subsamples per replicate (32 fruits per variety). Fruits were wound inoculated with a $10^6$ spores/ml suspension and were incubated at 68°F and 90% humidity for 5 days. Disease severity was measured as decay lesion diameter.

There were significant differences between different varieties (**Fig. 3A**). In general, white flesh varieties tested in this experiment were more resistant to sour rot than yellow flesh varieties (**Fig. 3B**). The most susceptible varieties were 2 yellow varieties with a lesion size of 17.3 and 18.3 mm, respectively. The most resistant varieties developed lesions with less than 3 mm in diameter. Gil et al. (2002) compared yellow and white flesh cultivars for their content of phenolic compounds and other chemicals present in the peel and flesh and found significantly high phenolic compounds in some varieties (such as Snow King and Bright Pearl), which were tested by us for sour rot susceptibility and found them to be more resistant to sour rot than Spring Lady which had significantly lower amounts of phenolics. They also found that titratable acidity was higher in yellow flesh than white flesh peach cultivars. Prusky (1996) reviewed pathogen quiescence in post harvest diseases and discussed fruit factors and how high acidity in unripe fruits and high phenolics could contribute to fruits being less susceptible to certain postharvest diseases. On the other hand, Prusky and Lichter reviewed the effect of pH on the expression of fungal virulence factors such as the enzyme polygalactorunase. Acidic environments enhanced the expression of polygalactorunase genes in fungi such as *Botrytis cinerea* and *Penicillium expansum*. Polygalacturonase expression was high at pH 4, but then was minor at pH 5. This may explain why yellow varieties (which are more acidic than the white varieties) were more susceptible to *G. candidum* which produces polygalactorunase, too. The sour rot susceptibility study suggests that special care is needed when harvesting and handling the yellow varieties to minimize any physical damage that may cause any injuries resulting in an increase in disease severity, and also to closely monitor those varieties during fruit preconditioning.

Because high relative humidity is a critical factor for the development of sour rot decay, in 2007, we designed and experiment to test the effect of different relative humidities (100%, 99%, 85%, and 75%) created by various salt solutions (Winston & Bates, 1960) in sealed plastic container. Fruits were surface sterilized and inoculated with spore suspension as described above. Fruits were incubated at 68°F.

There was no significant difference between different relative humidities. The highest disease severity was at 100% and 99%, and then dropped as expected as the relative humidity dropped to 85%, and increased unexpectedly as the relative humidity decreased to 75%. This inconsistency in disease severity might be due to the fungus acquiring humidity and water necessary for its growth directly from the saturated fruit tissues via the wound. To test this theory again, fruits will be inoculated without wound, and it is of relevance to test the effect of different relative humidity on spore germinations and growth and infection of unwounded fruit.

**3. Determine whether *G. candidum* multiplies in the packinghouse.**

A packing line in each of seven packing houses was sampled six times during the 2007 season. Samples were taken by sampling randomly the surfaces of the line at different locations (**Fig. 4**) using Rodac plates containing potato dextrose agar amended with novobiocin (Nov-PDA) and supplemented with fludioxonil. The locations where sampling was done included the fruit dumping location, the brushes, belt after the brushes, and finally the fruit sorting tables.

Propagules of *G. candidum* were detected on all four areas sampled in five packinghouses out of seven (**Fig. 5A**, **B**, **C**, **F** & **G**) at some point during the whole period of sampling. In three packinghouses where good sanitation measures were applied, the frequency and *G. candidum* population was less than in other packinghouses (**Fig. 5C**, **D** & **E**). In those three packinghouses, the packing line was cleaned every day after operation. These data confirmed the 2006 data and suggested that the brushes may create minute wounds that allow nutrients from the fruit tissues to leak and support the growth and reproduction of *G. candidum* and "inoculation" of the fruits can occur in this way. The results also show that whenever good sanitation practices are taken, then the frequency and numbers of *G. candidum* will be reduced, and thus the risk to have a sour rot problem in the packing house will also be reduced.

We also collected solution that was drained after washing the fruit in the brush area to determine if these liquids have enough nutrients (i.e., sugars, vitamins, etc.) may support growth and reproduction of *G. candidum.* The solution was collected from one packinghouse in a sterile container, stored at 36 to 39°F, and used either the same or the day following its collection. An aliquot of 100 µl of the solution were plated on semi-selective media at time 0; then the solution was kept on a rotary shaker at 100 rpm and 77°F and then plated again every 24 hours for 72 hours. Any increase in the *G. candidum* propagules numbers will support the theory that *G. candidum* has the ability to multiply in this solution.

There were no *G. candidum* propagules growing on the plates when the solution was plated immediately after collection or after shaking in the rotary shaker. In a previous year, we could detect *G. candidum* propagules in the solution, but no propagules were detected in the 2007 sampling. It might be that fruits were cleaner during the 2007 season and they did not bring a lot of propagules of *G. candidum* to contaminate the wash solution. It seems that when fruit are handled properly in the field and picked at proper maturity, and they are free of defects, it may be possible to reduce the number of fruit that can be damaged by the packing system without leaving any plant residues and fruit juice on the brushes. Another explanation could be the use of Mentor® fungicide as a postharvest treatment has a major effect on *G. candidum* propagules present in the solution.

**4. Identify sanitation practices in harvest equipment and the field which can reduce sour rot decay.**

*Compare orchards with tillage and non-tillage*: In 2007 we could not locate fields with tillage to compare orchards with tillage and non-tillage. In 2007 we repeated the experiment from 2006 to evaluate the distribution and the numbers of *G. candidum* propagules in the soil, on the leaves, and fruit surface in non-tilled orchards. Leaves, fruits, and soil samples were collected. Three composite soil samples were collected from the top first inch per field from 43 peach and nectarine orchards from Fresno, Kings, and Tulare counties. Twenty five leaves and 30 fruits collected per orchard were washed with water and surfactant. The washings were plated in plates containing Novobiocin-amended PDA (Nov-PDA) supplemented with 1 ppm fludioxonil, using the Spiral Plater to enumerate the *G. candidum* propagules on leaf and fruit surface. A composite soil sample of representative fields with different propagules levels of *G. candidum* and soils of fields that did not yield any *G. candidum* were submitted to the UC Analytical Laboratory for organic matter analysis. To determine disease incidence in cull fruits and in fruit boxes, ten boxes of fruits were harvested directly from five fields (#56, #57, #59, #62, and #63); the fruits were not treated with any fungicides, placed in industry's standard cardboard boxes, and incubated at 68ºF and >90% relative humidity for 5 days when incidence of fruit with sour rot was recorded. In addition, we recorded the number of boxes showing at least one decayed fruit with sour rot. Also cull fruit from the same fruit lots that were treated with Scholar® and Mentor® in the packinhouse were collected and evaluated for sour rot as described earlier.

*G. candidum* was detected on the leaves in 7.1% of the fields and on the fruit surface in 3.6% of the fields sampled. *G. candidum* was detected in soils of 44.2% fields sampled (**Table 1**). These results confirmed the 2006 results that soil is a major source of inoculum of *G. candidum* that can reach the canopy (leaves and fruit) and move to the packing house on plant material or harvesting equipment.

Sour rot developed only in fruit of one field collected directly from the field without running them through the packing line. The percentage of the fruit with sour rot from that field was 0.2% and sour rot was present only in 10% of the boxes. On the other hand, sound cull fruit developed sour rot after running fruits from the same lots through the packing line for all sampled fields

(**Fig. 6**). The percentage of fruit decayed with sour rot ranged between 1 and 19.7%. This result confirms that the packing line is a major source of inoculum and can “inoculate” fruit through creating micro wounds and contaminating fruit that are sour rot free. These cull fruit were sorted out after they have been treated with Scholar® and Mentor®. There is no report or any evidence that *G. candidum* growing on treated fruit are resistant to these newly registered fungicides, or if the infection already started before the fruit were treated in the packinghouse. Because a Mentor® application may also be applied in the field for the control of other stone fruit diseases (i.e., brown rot) prior to the treatment in the packinghouse, it is likely that there may be pressure for the selection of resistant isolates of *G. candidum*. Therefore, there is a possibility for resistant population to emerge in the field or the packinghouse. Screening isolates of *G. candidum* for resistance to these fungicides could provide the stone fruit growers an advantage to make decisions on resistance management. Currently, a number of isolates of *G. candidum* are being checked for resistance to Scholar® and Mentor® fungicides.

*Effect of soil depth***.** Soil samples were collected from five fields at three soil depths 1, 2, and 4 inches deep and population levels of *G. candidum* were determined as described earlier. In 2006 we recovered propagules of *G. candidum* in soil as deep as 4 inches. In 2007 we recovered propagules also as deep as 4 inches. The highest population was in the top 1 inch and decreased significantly as the depth increased. This result suggests that *G. candidum* population decreases as soil depth increases due to non-tillage practices that allow the accumulation of fruit and plant residues on the surface, resulting in higher population of *G. candidum* on the surface. Also this result raises the questions whether *G. candidum* propagules can survive deep in the soil. If *G. candidum* does not survive well deeper in soil than on the surface, plowing the field once in while could serve as a cultural practice to reduce the propagules of *G. candidum* in soil.

## CONCLUSIONS

1. Main source of inoculum of sour rot decay caused by *Geotrichum candidum* of peach and nectarine is the orchard soil brought in the packinghouse as dust on the fruit surface and occasionally on leaf debris.

2. No definite conclusions can be made regarding reproduction of *G. candidum* in the packinghouse, although *G. candidum* could be detected in wash water in one year and not in 2007.

3. Based on the 2005, 2006, and 2007 results, *G. candidum* propagules can contaminate the packing line particularly at the fruit dump area and the area at the brushes and after the brushes and “inoculate” fruit that originated from orchards whose soil and fruit were free of any *G. candidum* propagules.

4. The species *Geotrichum citri-aurantii*, whish attacks citrus, is as pathogenic as *G. candidum* to nectarines and produces polygalacturonase comparable to highly pathogenic isolates of *G. candidum*.

5. Isolates of *G. candidum* from different substrates produced pectinolytic enzymes (polygalacturonase) that are considered pathogenicity factors and can be similar to those produced by bacteria and other fungi causing soft rots on different agricultural commodities. Differences in the production of polygalacturonase could account for differences in pathogenicity since there was a trend between enzyme production and disease severity.

6. Peach and nectarine cultivars vary in their susceptibility to sour rot. In general, white flesh varieties are less susceptible to sour rot than yellow flesh. More care is needed when handling and processing sensitive cultivars to reduce injuries to fruits.

7. Significant development of sour rot on cull fruit resulted mainly from contamination of the packing line with *G. candidum* propagules. These fruits were also treated with Mentor®, and the resistance of *G. candidum* to this fungicide is not known yet. Isolates are now being screened to determine if there are any resistant isolates that may have emerged in 2007. Screening isolates for resistance to Mentor® and/or Scholar® in a timely fashion will be an advantage to growers to make decisions on resistance management as needed.

8. Populations of *G. candidum* decrease as soil depth increases. If *G. candidum* did not survive in deep soil as well as on the soil surface, then plowing the soil once in while would result in reducing the propagules of the pathogen in the orchard. More studies are needed on this area of investigation.

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**Table 1.** *Geotrichum candidum* population on the surface of leaves, fruits, and soil of commercial orchards in 2006 and 2007.

| Field | Leaf surface (propagules/ leaf) | Fruit surface (propagules/ fruit) | Soil (propagules/ gram) | Leaf surface (propagules/ leaf) | Fruit surface (propagules/ fruit) | Soil (propagules/ gram) |
|---|---|---|---|---|---|---|
| | 2006 | | | 2007 | | |
| 5 | 100 | 0 | 244.4 | 0 | - | 0 |
| 6 | 0 | 0 | 0 | 1.64 | - | 16.7 |
| 7 | 0 | 0 | 44.4 | 0 | 0 | 1766.7 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 77.8 |
| 10 | 0 | 0 | 0 | 0 | - | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 44.4 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | - | 0 |
| 23 | 0 | 0 | 0 | 0 | - | 0 |
| 25 | 0 | 0 | 0 | 0 | - | 0 |
| 27 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 256 | 0 | 0 | 0 | - | 105.6 |
| 29 | 0 | 0 | 88.9 | 0 | 0 | 0 |
| 31 | 5 | 67 | 377.8 | 0 | 0 | 0 |
| 32 | 0 | 0 | 155.6 | 0 | - | 0 |
| 33 | 0 | 0 | 0 | 0 | - | 0 |
| 34 | 0 | 0 | 0 | 0 | - | 0 |
| 35 | 0 | 0 | 200 | 0 | - | 0 |
| 36 | 0 | 0 | 1200 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 0 | 255.6 |
| 39 | 0 | 0 | 555.6 | 0 | 0 | 55.6 |
| 41 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 66.7 | 0 | 0 | 44.4 |
| 43 | 0 | 0 | 133.3 | 0 | 0 | 27.7 |
| 44 | 0 | 0 | 22.2 | 0 | - | 1344.4 |
| 45 | 0 | 0 | 22.2 | 0 | 0 | 27.8 |
| 46 | 0 | 0 | 0 | 0 | 0 | 27.8 |
| 47 | 0 | 0 | 333.3 | 0 | 0 | 16.6 |
| 48 | 0 | 0 | 2155.5 | 0 | 0 | 1177.8 |
| 49 | 0 | 0 | 866.7 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | 0 | 15 | 22.2 |
| 51 | 0 | 0 | 17111.1 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 0 | 0 | 0 | 0 | 0 | 1127.8 |
| 54 | 323 | 593 | 22.2 | 0 | 0 | 0 |
| 56 | 18 | 0 | 66.7 | 0 | 0 | 194.4 |
| 57 | 116 | 0 | 133.3 | - | 0 | - |
| 59 | 0 | 0 | 3333.3 | 48 | 0 | 2383.3 |
| 60 | 8 | 0 | 111.1 | 0 | 0 | 0 |
| 62 | - | - | - | 3.3 | - | 455.6 |
| 63 | - | - | - | 0 | - | 44.4 |
| 64 | - | - | - | 0 | - | 0 |

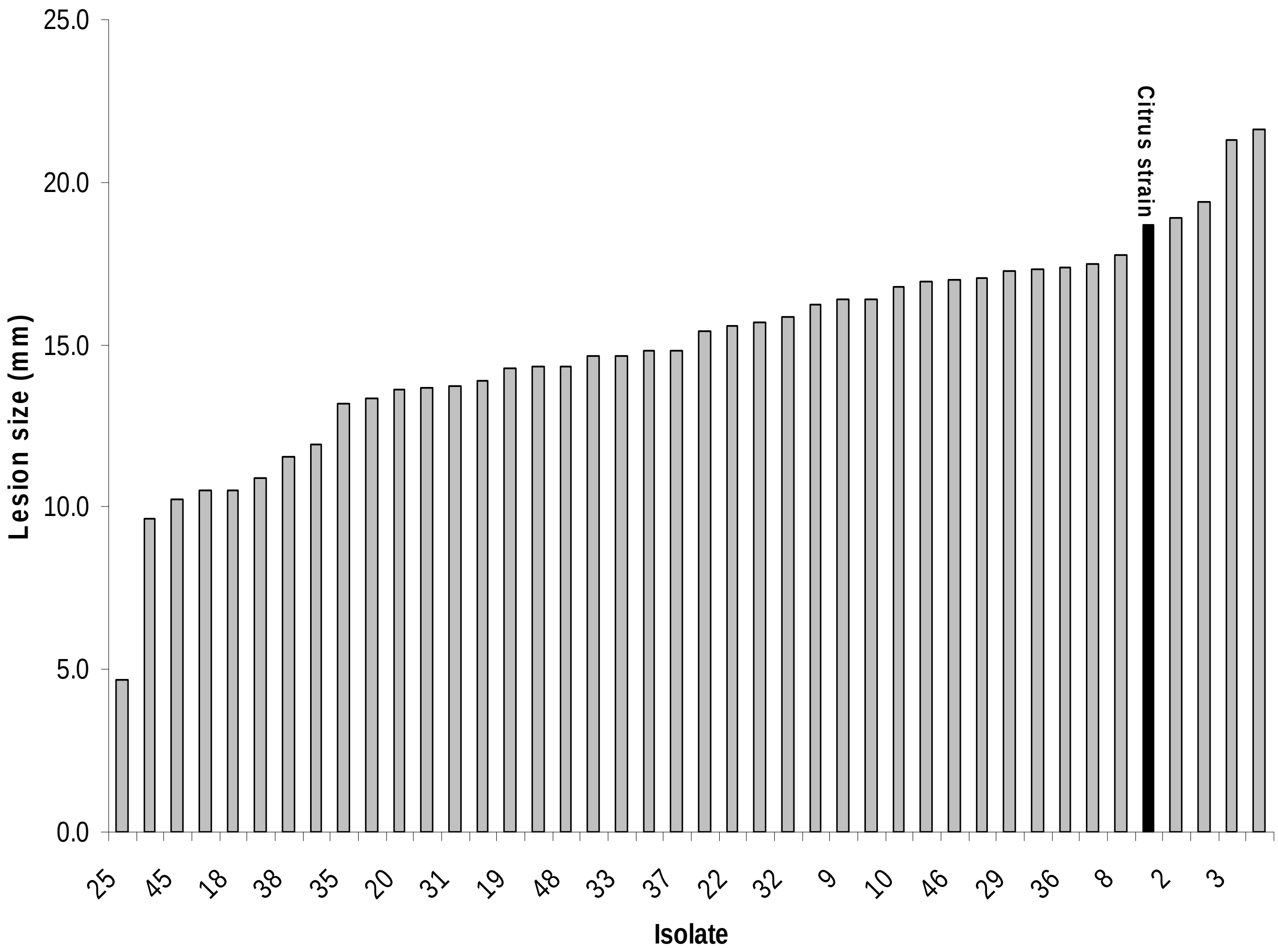



**Figure 1.** Pathogenicity and disease severity of 41 isolates of *Geotrichum candidum* isolated from leaves, fruit surface, soil, decayed nectarines and peaches, tomato, and from packing lines in commercial packinghouses.

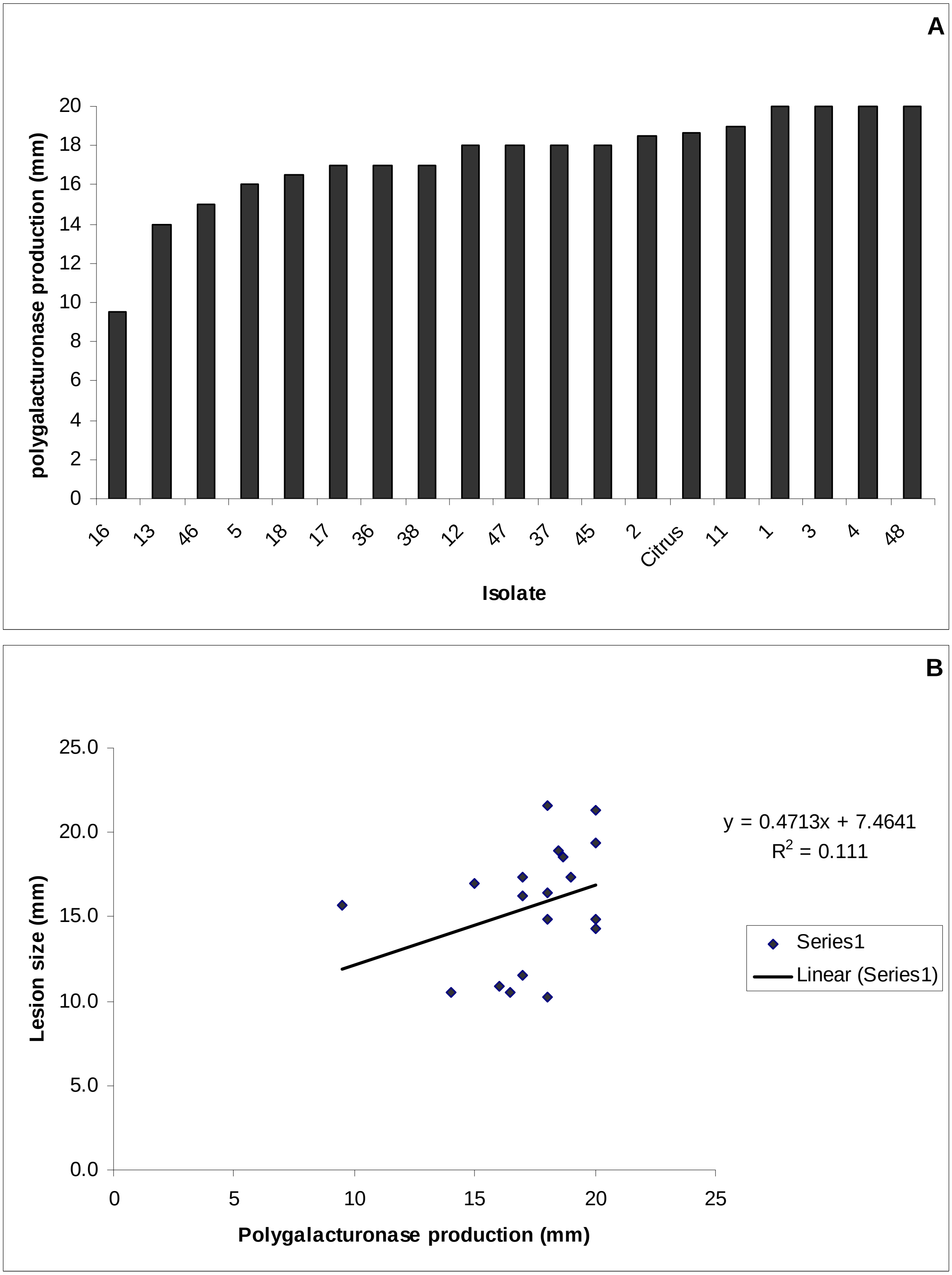



**Figure 2. A,** Production of polygalacturonase from 18 different isolates of *Geotrichum candidum* and one isolate of *G. citri-aurantii*. **B,** Correlation between polygalacturonase production (measured as the diameter of the clear area of the polypectate medium in a cup-plate assay) and lesion size.

A

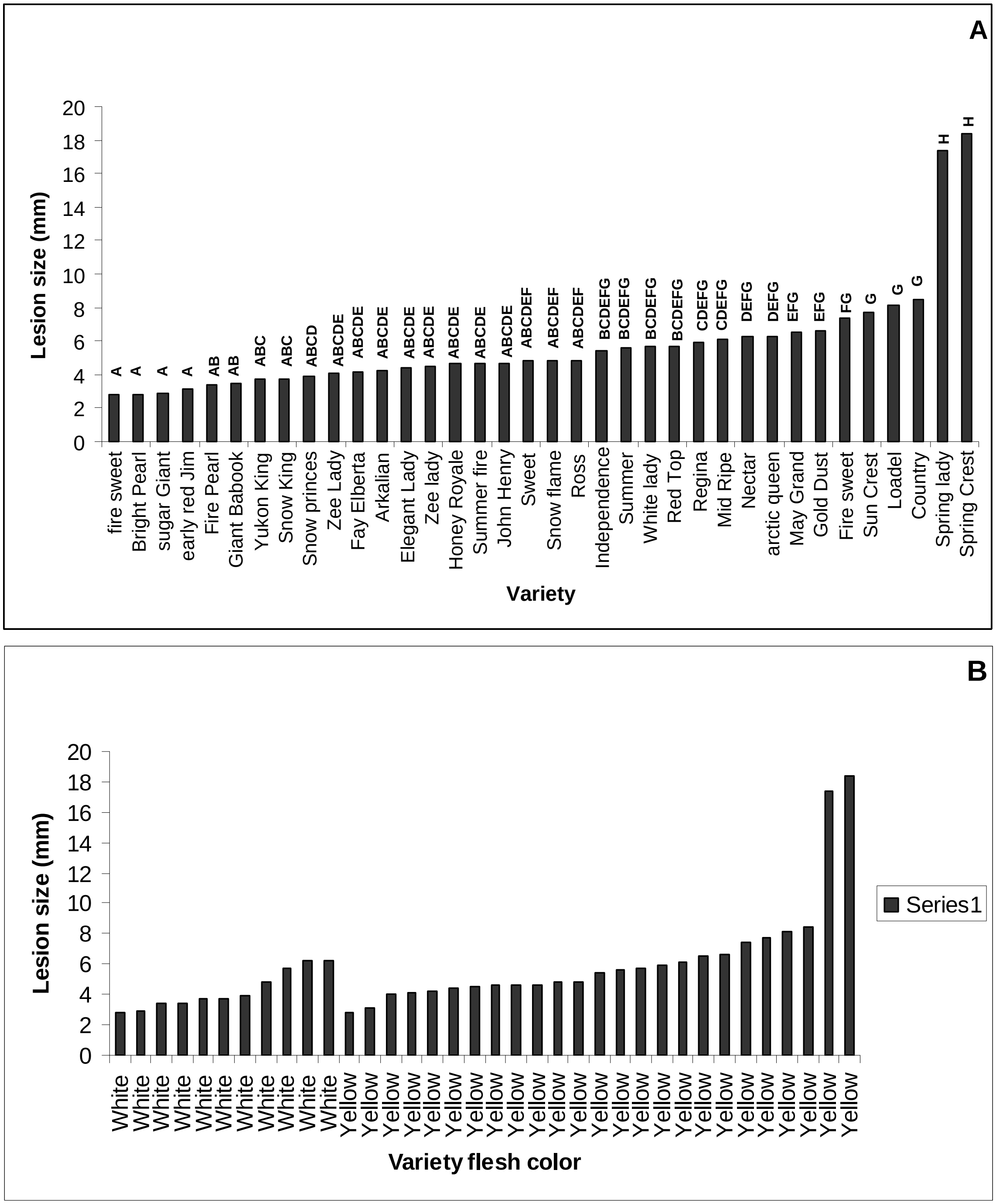



**Figure 3. A,** Sensitivity of different varieties to sour rot caused by *Geotrichum candidum;* and **B,** Sensitivity of different varieties arranged by their flesh color.

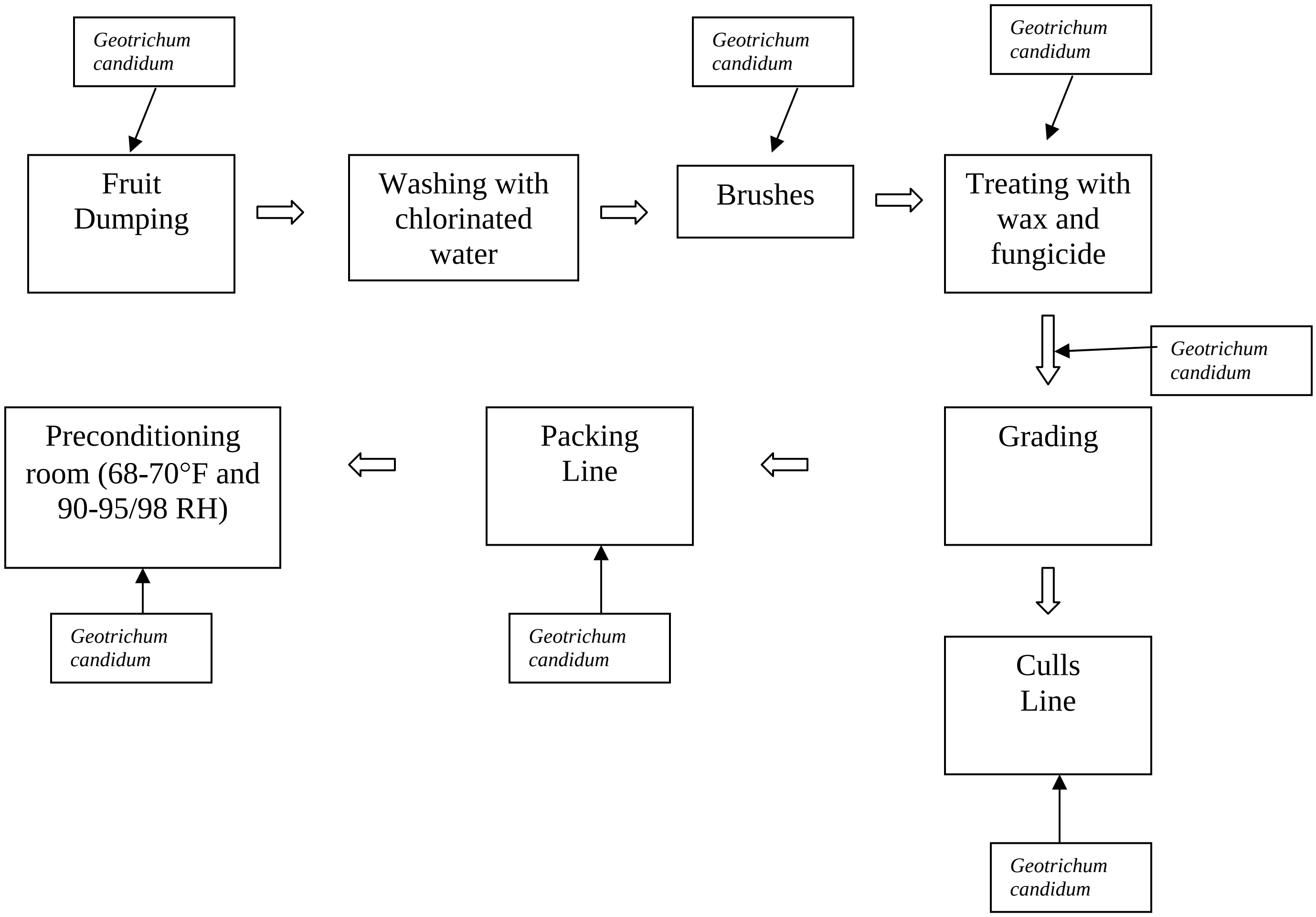
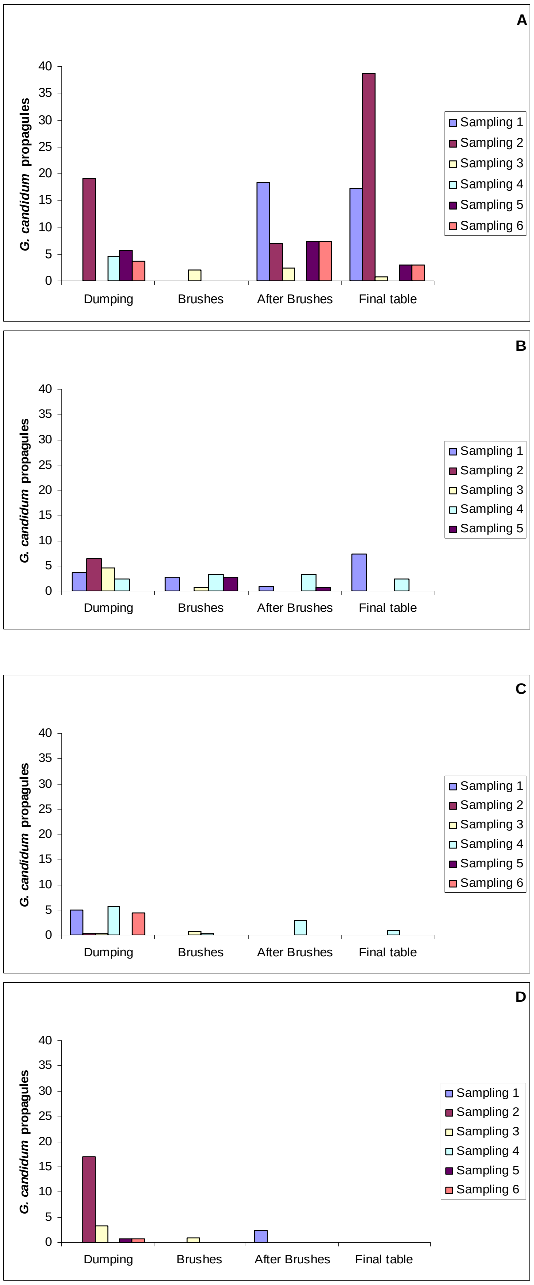



**Figure 4**. A diagram showing the areas of a packing line in a packinghouse where *Geotrichum candidum* propagules was recovered.


A
G. candidum propagules
40
35
30
25
20
15
10
5
0
Dumping
Brushes
After Brushes
Final table
Sampling 1
Sampling 2
Sampling 3
Sampling 4
Sampling 5
Sampling 6
B
G. candidum propagules
Dumping
Brushes
After Brushes
Final table
Sampling 1
Sampling 2
Sampling 3
Sampling 4
Sampling 5
C
G. candidum propagules
Dumping
Brushes
After Brushes
Final table
Sampling 1
Sampling 2
Sampling 3
Sampling 4
Sampling 5
Sampling 6
D
G. candidum propagules
Dumping
Brushes
After Brushes
Final table
Sampling 1
Sampling 2
Sampling 3
Sampling 4
Sampling 5
Sampling 6

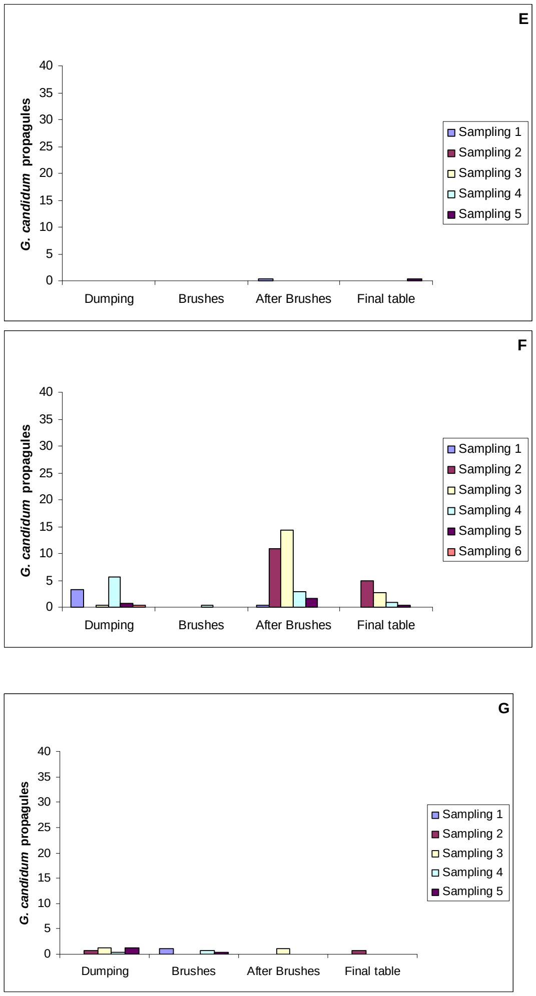
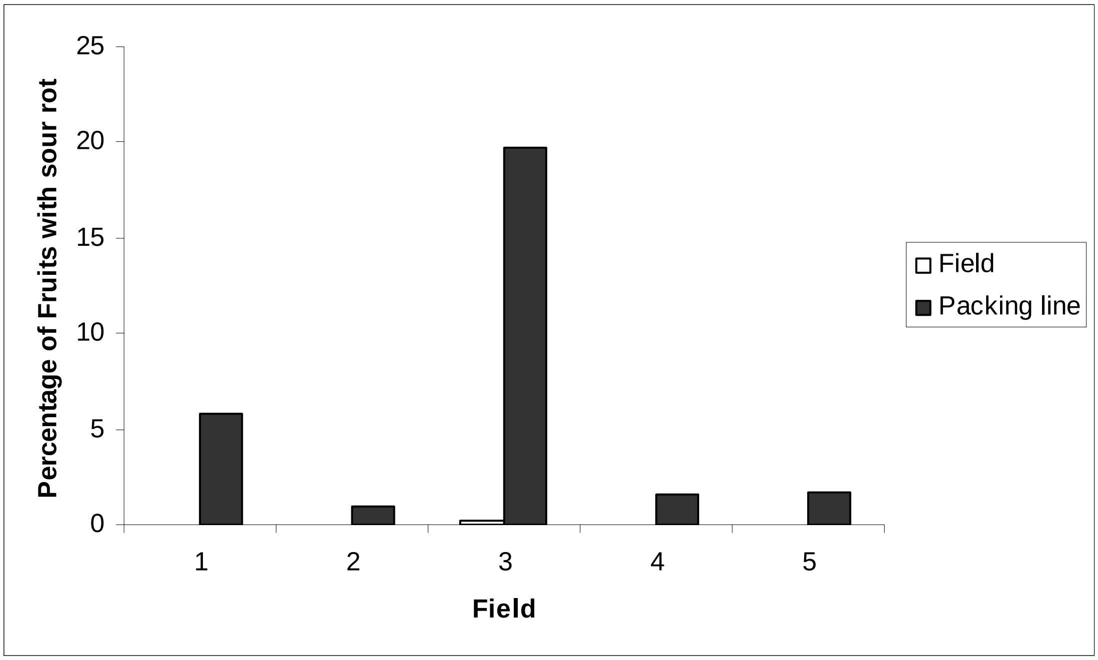



**Figure 5.** *Geotrichum candidum* propagules per plate after sampling four times from various locations along the packing line in each packinghouse. Packing line B, E, and G was sampled only five times.



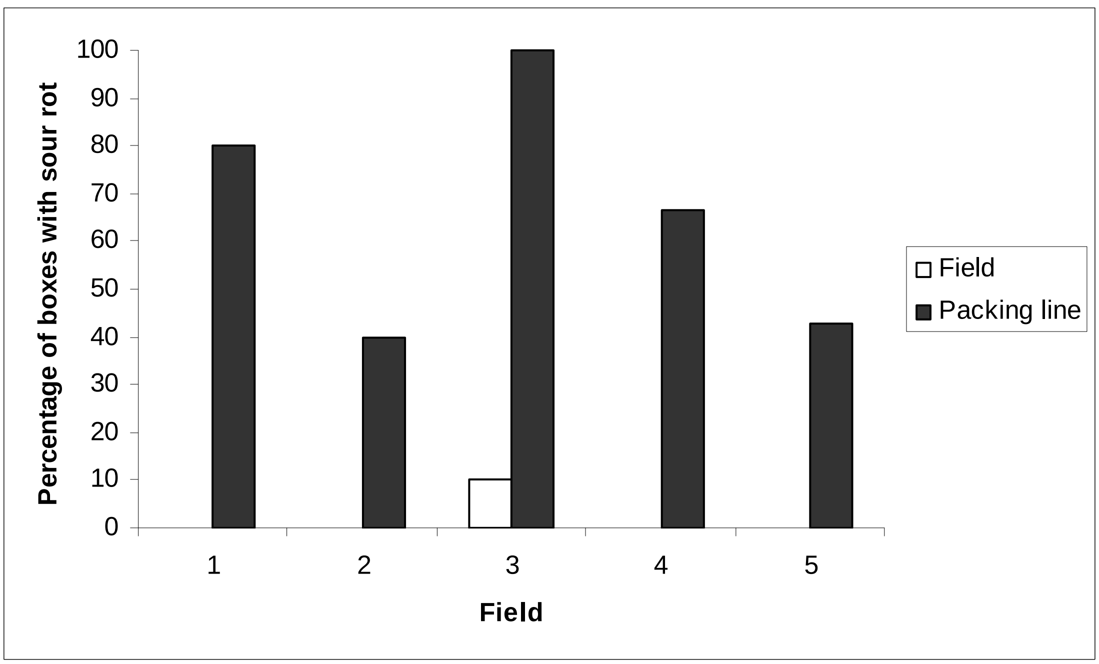




**Figure 6.** Percentage of fruits, and boxes with sour rot after collecting them directly from the field (white bars) or after processing through the packing line (black bars).

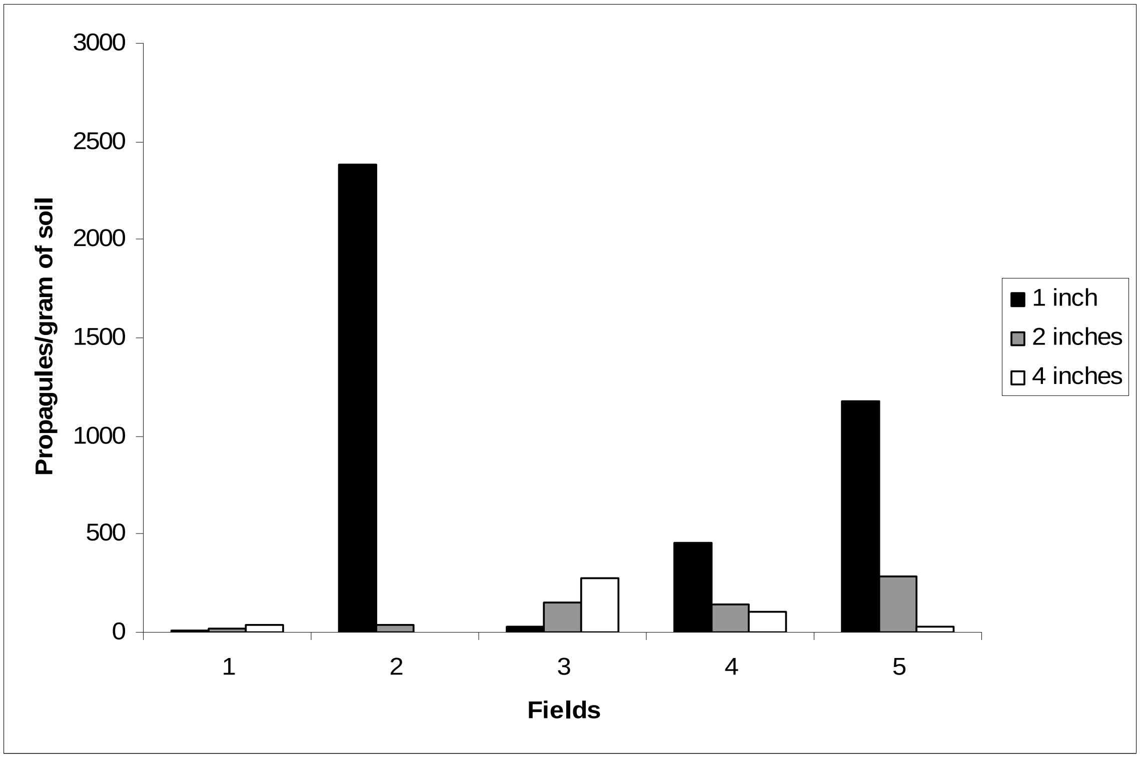



**Figure 7.** Soil population of *Geotrichum candidum* at different soil depths in five different orchards.