Biology, Epidemiology, and Management of Sour Rot Complex Decay in Stone Fruit

PROJECT LEADERS: Dr. Themis J. Michailides
COOPERATORS: M. Yaghmour, R. Bostock, Dr. C. Crisosto, Dr. J.E. Adaskaveg, D. P. Morgan, and H. Reyes

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 2001-2006.

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 the 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 more frequently isolated, all the inoculation and transmission experiments were done using *G. candidum* isolates.

During 2005 and 2006 significant progress was made in understanding the biology of the main cause of sour rot (*G. candidum*) in stone fruit orchards and the various factors affecting the disease in stone fruit orchards. Specifically, the objectives in 2006 were:

Objectives

1) To determine pathogenicity of isolates of *G. candidum* collected from stone fruit, other kind of fruit, tree canopy, soil, and packinghouse.
2) To determine the sources of inoculum in orchard soils, the time when fruit become infected, and orchard and cultivar factors affecting sour rot incidence.
3) Determine what role orchard insects play in transmitting the sour rot pathogens in stone fruit.

Methods and Results

1. To determine pathogenicity of isolates of *Geotrichum candidum* collected from stone fruit, other kind of fruit, tree canopy, soil, and packinghouse.

To test for pathogenicity, isolates of *Geotrichum candidum* were isolated from soil, decayed fruit, packing lines, and surfaces of peach leaves and fruits. Single spore isolates were grown on acidified potato-dextrose agar for 72 hours and spore suspensions of 10^6 spores per milliliter suspension were prepared to inoculate nectarine fruits. Forty one isolates representing all sources of inoculum were tested in a complete block design with four replicated fruit per isolate. 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 on a raised plastic mesh, placed in plastic containers, and water was added to the bottom of the containers to create a high humidity. Fruits were wounded and inoculated with 20 µl of 10^6 spores/ml of each isolate. The plastic containers were incubated on a laboratory bench. Pathogenicity and lesion diameter were recorded after five days of inoculation. Data were subjected to the arcsine transformation to meet the assumptions of the ANOVA.

Forty one isolates of *Geotrichum candidum* representing isolates collected from different substrates, such as soil, fruit surface, decayed fruit, and leaf surface were tested for pathogenicity on stone fruit. In addition, isolates from tomato fruits were also used in this experiment. All isolates were found to be pathogenic on peaches and nectarines. Also severity of the isolates was determined by measuring the lesion size that ranged from 4.7 mm up to 21.7 mm in diameter **(Fig. 1)**. Severity of the isolates was not statistically different from each other. The results suggest that a wide range of *G. candidum* strains exist in stone fruit orchards and can be equally pathogenic in decaying stone fruit. Therefore, particular care should be taken when stone fruit orchards of susceptible cultivars are established in fields that were cropped previously with tomatoes or other susceptible crops plants. Even though severity between isolates was not statistically different, the range of severity among the *G. candidum* isolates suggests that there might be differences in production of such pathogenicity factors as pectinolytic enzymes (polygalactoronases).

Objective 2. To determine the sources of inoculum in orchard soils, when fruit gets infected, and the orchard and cultivar factors that affect sour rot incidence.

Leaves, fruits, and soil samples were collected. Three composite soil samples were collected from the top first inch per field from 48 peach and nectarine orchards from Fresno and Tulare counties. Twenty five leaves and 30 fruits 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. To determine disease incidence in cull fruits and in fruit boxes, fruits from fields 5 to 55 were placed at 68ºF and >90% relative humidity **(Table 1)** for 5 days when incidence of fruit with sour rot was recorded. In addition, we recorded the number of boxes with at least one sour rotten fruit out of the total boxes used. Ten boxes of fruits were harvested directly from the

Field 56, 57, 58, 59, 60, and 61, without running the fruits through the packing lines. These fruits were incubated and sour rot incidence was recorded in the same way as described earlier.

For the orchards where cull fruits were collected, only six orchards yielded *G. candidum* from leaf washings and only three orchards of these yielded the pathogen on both leaves and fruit. *G. candidum* was detected only in the soils of 21 out of the 41 orchards. The levels of propagules ranged from as low as 22 and high as 17,111 propagules per gram of soil.

Fruit collected from the field remained free from *G. candidum* except fruit from two orchards with very low disease incidence of 0.8% and 1.43 %, respectively, after incubating under conditions conducive for sour rot development. However, 0.8% to 14.7% of the cull fruit collected from eight fields was infected with sour rot, and 33.3% up to 100% of the boxes containing cull fruits had infected fruits with sour rot. Fruit from four orchards (#7, #29, #40, and #61) developed sour rot although propagules of *G. candidum* were not detected in the soil and the fruit and leaf surface of these orchards, except in orchard #40 in which propagules were detected on the surface of fruit and leaves. These results suggest that a major source of contamination is in the packing house and probably propagules of *G. candidum* can be brought into an orchard even when the pathogen is not present in the soil of the orchard. Surprisingly, there was no correlation between the levels of propagules of *G. candidum* in soil and the propagules recovered on leaves and fruit neither with the amounts of sour rot in those fields for which sour rot was determined.

Objective 3. To determine what role orchard insects play in transmitting the sour rot pathogens in stone fruits.

Also we surveyed three sites where cull fruit were dumped after being taken from the packing houses. In one site, the fruit were dumped on the road next to a peach orchard and it seemed that vehicles drove over them several times. This site had a high incidence of fruit with sour rot usually developing on the surface of culls touching the soil, and millions of nitidulid and vinegar flies. In the second site which was close to the packinghouse and a young stone fruit orchard, we were not able to find sour rot on fruit because the mummies were disked into previously dumped fruit that were very dry. The fruit in this site dried quickly and did not allow the development of any sour rot (safer way of dumping cull fruit). The third site was similar to the first site with a lot of fruit with sour rot and abundance of nitidulid beetles and vinegar flies. In the fruit dump sites where sour rot developed, up to 30% of fruit were infected with sour rot, mainly on the side of fruit touching the soil.

Both dry fruit beetle and fruit flies were collected from these fruit dump sites, brought to the laboratory, killed by freezing, and 101 dry fruit beetles, and 54 fruit flies plated on Nov-PDA supplemented with 1 ppm fludioxonil without surface sterilization. The plates were incubated at 23ºC (about 73ºF) for 5 days and the number of insects carrying *G. candidum* propagules was recorded. To determine whether the insects carried the propagules inside their gut system, 70 dry fruit beetles and 40 fruit flies were collected from the same fruit dump sites, surface sterilized with 10% bleach (0.1% chlorine solution) for 30 seconds, and plated on Nov-PDA amended with 1 ppm fludioxonil. Data were analyzed as categorical data.

Twenty five percent of the dry fruit beetle collected from the field carried *G. candidum* propagules on their body and was significantly higher from the beetles that were surface sterilized (only about 3% yielded *G. candidum* colonies). This suggests that the dry fruit beetles carry *G. candidum* propagules mainly on the surface of their bodies and can disseminate *G. candidum* propagules from infected fruits or culls in the field to healthy fruits before harvest. Similar results were obtained by plating fruit flies **(Table 2)**.

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

Soil and leaf samples were collected from a field with tillage and were analyzed for propagules levels of *G. candidum* as described earlier. In this orchard an average of 3 propagules of *G. candidum* were detected on the leaves but no propagules were found in the soil **(Table 1)**. To determine the density of *G. candidum* as affected by soil depth, soil sample were taken at 1, 2, and 4 inches from a field with history of sour rot. The soil suspension were plated in Nov-PDA plates supplement with 1 ppm Novobiocin and incubated at 23°C (73°F) for 3 days. *G. candidum* was isolated from the 1 and 4 inches depth at levels of 267 and 111 propagules per gram of soil, respectively.

The packing line in each of four packing houses was sampled four times during the 2007 season. Samples were taken by sampling randomly the surfaces of the line at different locations (Fig. 2) using Rodac plates containing Nov-PDA supplemented with fludioxonil. The locations where sampling was done included: the fruit dumping location, the brushes, the belt after the brushes, and the final sorting tables. Sour rot propagules were detected on four locations of the packing line with the highest propagules recovered from the brushes and the area after the brushes **(Fig. 2)**. The results suggest that the brushes may create minute wounds that allow nutrients from the fruit tissues to leak and support the growth and propagation of *G. candidum.* In addition, "inoculation" of the fruit with propagules of the sour rot pathogen(s) can occur in this way. This is also an indication that the brushes may redistribute propagules of *G. candidum* in other fruit by removing them from fruit with sour rot lesions. In previous research we showed that sometimes fruit can develop sour rot lesions while they are on the tree. Furthermore, propagules of the pathogen can exist in the dust that is present on the surface of fruit and leaves.

Since contamination and "inoculation" of fruit can occur at the packing line, sanitations measures to clean the line and specially the brushes is an important measure to reduce "inoculation" of the fruits by sour rot spores present on the brushes. Also, it will be important that the brushes are checked from time to time to make sure that they do not change stiffness over time and do not create wounds on the fruit.

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 and occasionally on leaf debris.

2. Fruit decayed by sour rot and left in the orchard or culls dump at the side of an orchard can serve as major source of enriching the soil with propagules and as sources of contamination of orchard insects.

3. Insect surveys in the field and previous studies on insect transmission show that insects such as dry fruit beetle and fruit fly can act as vectors to *G. candidum.* These insects carry the propagules of the pathogen passively because of external contamination.

4. No definite conclusions can be made regarding tilled orchards, although *G. candidum* can be found abundantly in the soil of non-tilled orchards. More orchards with tillage need to be sampled.

5. Based on the 2005 and 2006 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.

References

Förster, H., Kanetis, L., and Adaskaveg, J. E. 2004. Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus-fungicide interactions. Phytopathology 94:163-170.

Michailides, T. J., Morgan, D. P., and Day, K. R. 2003. First report of sour rot of California peaches and nectarines caused by yeasts. Plant Disease 88:222.

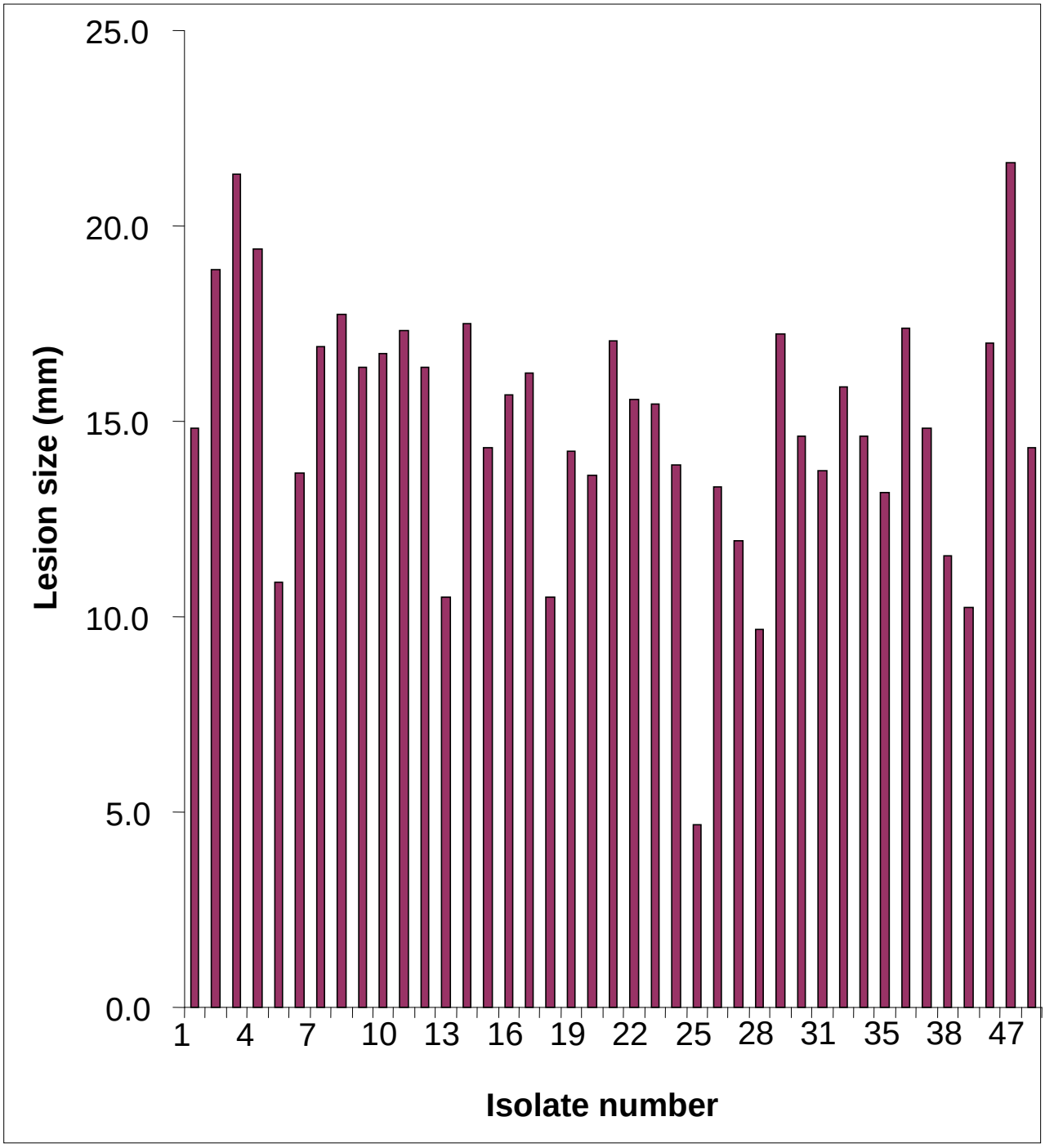

Figure 1. Pathogenicity and severity of 41 isolates of *Geotrichum candidum* isolated from leaves, fruit surface, soil, decayed nectarine and peaches, tomato, and packing lines in commercial packing houses.

Table 1. *Geotrichum candidum* population on the surface of leaves, fruits, and soil, and percentage of fruit and boxes with sour rot collected from a number of commercial orchards in 2006.

Field[1]	Leaf surface (propagules /leaf)	Fruit surface (propagules/fruit)	Soil (propagules/gram)	Percentage of fruits with sour rot	Percentage of boxes with sour rot
5	100	0	244.4	-	-
6	0	0	0	-	-
7[2]	0	0	44.4	14.7	83.3
8	0	0	0	-	-
9	0	0	0	-	-
10	0	0	0	-	-
13	0	0	600	-	-
19	0	0	0	-	-
20	0	0	0	-	-
21	0	0	44.4	-	-
22	0	0	0	-	-
23	0	0	0	-	-
25	0	0	0	-	-
27	0	0	0	-	-
28	256	0	0	-	-
29[2]	0	0	88.9	3.4	71.4
30	22	0	0	0.8	33.3
31	5	67	377.8	-	-
32	0	0	155.6	-	-
33	0	0	0	-	-
34	0	0	0	-	-
35	0	0	200	-	-
36	0	0	1200	-	-
38	0	0	0	-	-
39	0	0	555.6	-	-
40[2]	12	244	466.7	1.0	20.0
41	0	0	0		
42	0	0	66.7	5.1	85.7
43	0	0	133.3	-	-
44	0	0	22.2	-	-
45	0	0	22.2	-	-
46	0	0	0	7.1	100.0
47	0	0	333.3	-	-
48	0	0	2155.5	-	-
49	0	0	866.7	-	-
50	0	0	0	3.9	75.0
51	0	0	17111.1	-	-

52	0	0	0	-	-
53	0	0	0	-	-
54	323	593	22.2	-	-
55	0	0	111.1	-	-
56	18	0	66.7	-	-
57	116	0	133.3	-	-
58	20	0	0	-	-
59	0	0	3333.3	1.43	30
60	8	0	111.1	-	-
61[2]	0	0	0	0.3	10
Tillage	3	0	0	-	-

[1] Orchards 5 to 61 represent non-tilled peach and nectarine fields.
[2] Orchards with sour rot on the fruit but without propagules of *G. candidum* in the soil.

Table 2. Incidence of *Geotrichum candidum* on dry fruit beetles and fruit flies with or without surface sterilization and after plating them on Nov-PDA at 73°F for 5 days.

Species of insect	**% with *G. candidum***	
	Without surface[1] sterilization	**With surface[2] sterilization**
Dry fruit beetle	24.8	2.9
Fruit fly	25.9	0

[1] 101 dry fruit beetle and 54 fruit flies were plated.
[2] 70 dry fruit beetles and 40 fruit flies were plated.

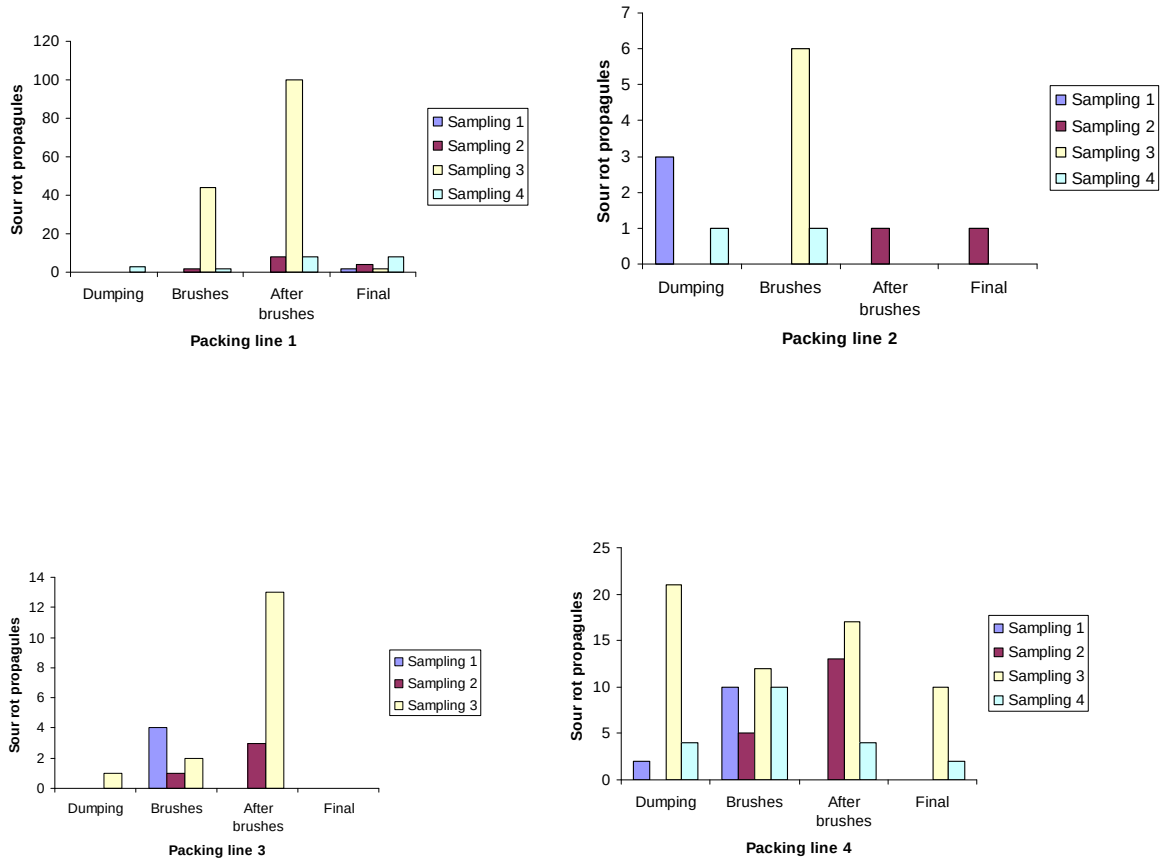

Figure 2. *Geotrichum candidum* propagules per plate after sampling four times from various locations along the packing line. Packing line 3 was sampled only three times.

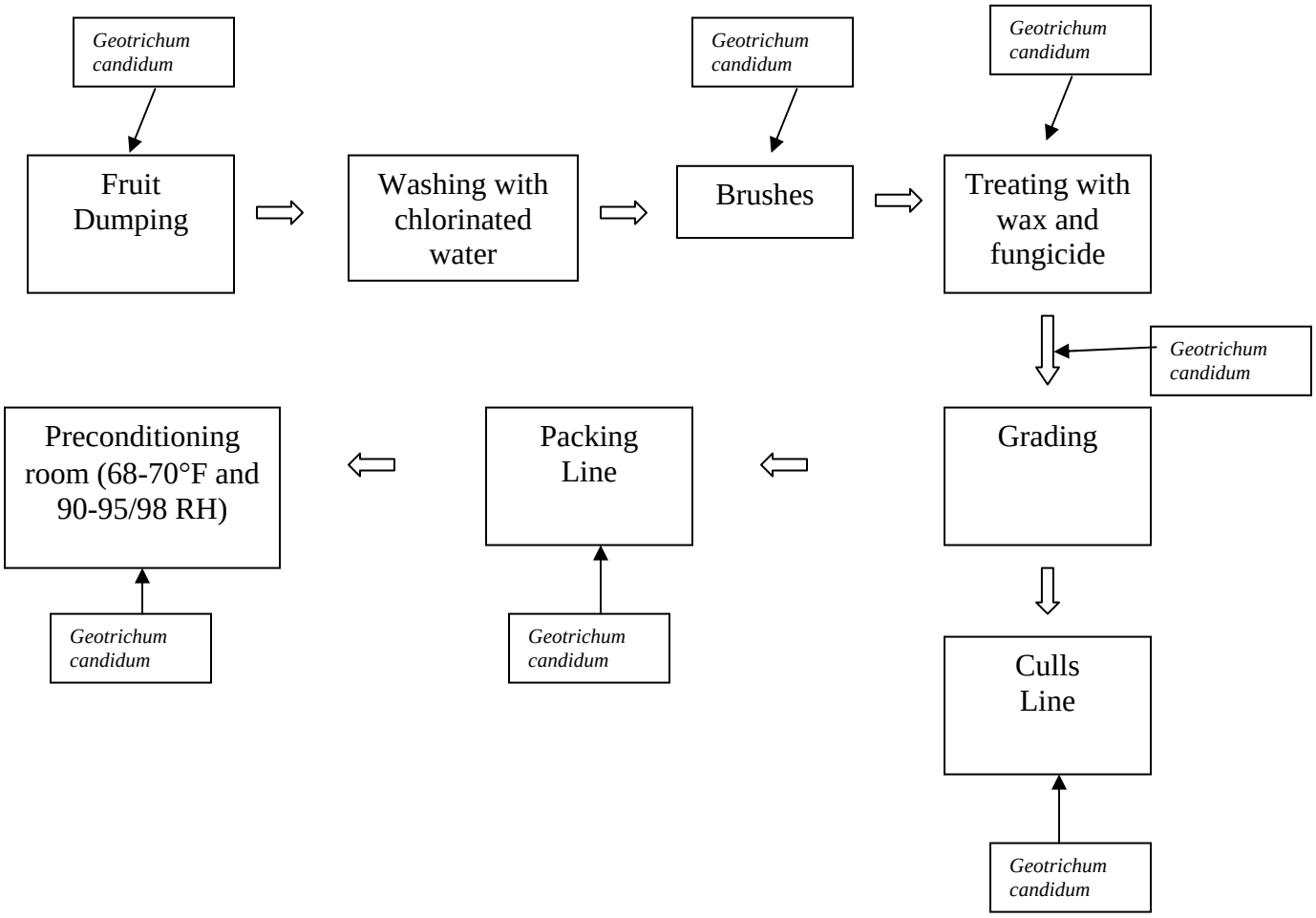

Figure 3. A diagram showing the areas of a packing line in a packinghouse where *Geotrichum candidum* propagules was recovered in sampling plates.