CONTROL OF POSTHARVEST BOX ROT OF PRUNES AND OTHER DISEASES

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OBJECTIVE 1. Complete studies on box rot of prunes and prepare manuscript for publication. (80%)

PROCEDURE. Manuscript enclosed.

OBJECTIVE 2. Brown rot blossom blight of prunes. (10%)

Brown rot fruit rot caused by Monilinia fructicola and Monilinia laxa is important to the prune industry as losses in crop yield, extra labor in removal of the decayed fruit or clusters after sorting, and reduction in grade by increasing the amount of deteriorated grade. Fruit rot cannot be controlled by application of fungicides during fruit maturation. Thus the only effective means other than insect control, during fruit maturation, is to reduce the numbers of blossom blight. Benomyl was introduced in 1972 and found to be more effective than captan yet did not control fruit russet scab; on peaches and nectarines we have a major problem with benomyl-resistant Monilinia fructicola emerging in 1977 and continuing to increase in both degrees of resistance and population levels. Although benomyl-resistant Monilinia spp. have not been detected in prune orchards, alternative fungicides have been researched and for the 1981 season triforine under the trade name Funginex^{R} has been approved to control brown rot blossom blight caused by benomyl-resistant Monilinia. Studies are in progress to learn more about the fitness of the benomyl-resistant Monilinia spp. and in testing and registration of new fungicides which will control them.

PROCEDURE AND RESULTS: For registration of triforine, tests on efficacy and residue were obtained and submitted for use in registration (in previous years) and finally in 1982 registration was approved through the help of the stone fruit industry and the legislature. Judging from the residual half-life of the chemical, we feel that the first spray at popcorn would be triforine and the second spray captan to control both brown rot and russet scab. For 1982, we anticipate testing in orchards with severe brown rot to detect benomyl-resistant Monilinia. The procedure for detection has been developed and the method submitted for publication by the Food and Agricultural Organization of the United Nations.

CONCLUSIONS: Triforine has been registered to control blossom bight. Other chemicals which appear equally or more effective such as Vangard and Prochloraz are being investigated to control brown rot blossom blight, russet scab, and green fruit rot.

OBJECTIVE 3. Leaf rust of prune. (5%)

Develop chemical control measures for leaf rust other than the only registered material sulfur.

PROCEDURE AND RESULTS: A test plot was designed which included 30 trees of prunes (French) in four rows. Ten trees, assigned randomly, were sprayed with Mancozeb (DITHANE M-45) in a dosage of 5 lbs/100 gallons of water and 10 trees with wettable sulfur (1.5 lbs/100 gallons of water). Ten trees were left unsprayed; they were used as control for this experiment.

Pustules of leaf prune rust (Tranzschelia discolor) were first observed after 20 days. Two rains occurred during the time between spraying and first reading for disease development. One hundred leaves (25 leaves/quarter/tree) were counted and the results were expressed in percentage of rusted/healthy leaves. Unsprayed trees had 7.3% rusted leaves; trees sprayed with wettable sulfur 6.7%; and Mancozeb 9.4% disease. Levels of disease between sprayed and unsprayed trees were not significantly different. A possible reason for this is the very low level of disease (lower than 10%) and high rainfall. Ninety-five percent of rusted leaves had developed only one pustule; the majority of the remaining 5% had 2 pustules, and a few leaves 3 or 4 pustules/leaf.

CONCLUSION: We were unable from these results to draw conclusions about the efficacy of the chemicals used because of the low level of the disease in our plot.

OBJECTIVE 4. Prune russet scab. (5%)

Prune russet scab is a problem if rain occurs during blossoming. Thus the occurrence of disease is serious one year and not the next or the disease is serious only in certain locations. The cause of the disease is unknown. Captan controls the russet scab yet we have no explanation. Studies are being made to understand why captan is effective and if other brown rot control fungicides are effective.

PROCEDURE AND RESULTS: Prune trees on the Davis Campus were sprayed every year with captan plus other chemicals so in the event of heavy disease incidence we will be able to obtain results. For the 1981 season we attempted to induce disease by irrigating during the blossoming period. The increase in disease was only slight.

Unless efforts are made to determine the cause of russet scab, we shall continue to have difficulties in evaluating fungicides for disease control.

draft for publication in PHYTOPATHOLOGY - 11/11/81
jmo

Relation of Postharvest Decay Fungi to the Deterioration of Dried French Prunes

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We thank B.T. Manji and E. Bose for technical assistance. This research was supported in part by a grant from the California Prune Advisory Board.

ABSTRACT

Sholberg, P. L., and Ogawa, J.M. 1981. Relation of postharvest decay fungi to the deterioration of dried french prunes. Phytopathology

Deterioration of dried French prunes expressed by soft, sticky macerated areas on the fruit and sometimes slippage of the skin was studied. The greatest amount of deteriorated fruit after drying was obtained by inoculation of fresh fruit with Rhizopus arrhizus and R. stolonifer; some deterioration also occurred with other fungi such as Aspergillus japonicus, Penicillium expansum, Mucor circinelloides, Cladosporium herbarum and Monilinia spp. Rhizopus spp. are the fungi most likely to develop on fresh prunes held for 24 hr or less after harvest and the most likely to be related to deterioration of dried prunes. The following supporting evidence was provided: 1) Rhizopus spp. were always observed before drying when significant amounts of dried fruit were deteriorated, 2) fresh prunes inoculated with Rhizopus spp. and incubated for 24 hr or more suffered severe

deterioration, and 3) naturally-deteriorated dried fruit obtained during commercial sorting and Rhizopus-inoculated fresh fruit that was dried contained polygalacturonase enzyme typical of that produced by Rhizopus spp. during decay of fresh fruit.

French prunes (Prunus domestica L. 'French') are at optimum maturity for harvest when the soluble solids content is at a minimum of 24 to 25% and the average fruit flesh fimness has decreased to 1.4 - 1.8 kg on the pressure gauge. Ripe French prunes remain in this optimum condition for harvest approximately 7 to 10 d (11). Mechanical shakers are used for harvesting the fruit which is caught in frames attached to the shaker or on canvas laid on the ground. Fresh fruit is harvested into bins of approximately 454 kg capacity and usually held less than 24 hr before being dried, but there are reports of fruit being held 48 hr or more when complications arise after harvest before dehydration.

The dehydration process (6, 15, 20) involves moving single layers of fruit in trays (0.9 m X 1.8 m) placed one above another on a four-wheeled car to form a stack approximately 2 m high. Loaded cars are then moved through a tunnel with a flow of hot air usually parallel to the trays for 12 to 20 hr. Every 105 to 135 min a truck of dried fruit is pulled from one end of the tunnel and a truck with fresh fruit is loaded into the other end. The maximum air temperature in the front end of the tunnel is about 90 C. The fruit itself is well below 90 C to prevent carmelization of sugars. Drying reduces moisture to approximately 20% and the dried prunes range from 38.4 to 51% reducing sugars

and 0.6 to 5.5% sucrose, both concentrations being unsuitable for mold growth (19). To equalize the moisture, dried prunes are placed in bins which are dumped every 3 to 4 d to redistribute the prunes. Eventually the prunes are taken to a processing plant where they are graded, processed and packaged for market.

Dried prunes can display damage in at least three distinctive ways. The first is the type where Miller and Tanaka (10) showed that at 20 C Aspergillus glaucus group fungi and Saccharomyces rouxii yeast could grow on dried prunes in equilibrium with a relative humidity of 76%. At higher temperatures and relative humidities they found that a wider range of fungi and yeast would grow on the dried prunes in a shorter period of time. Of those fungi which can grow on dried fruit, El Behadli (5) showed the most prevalent were Aspergillus glaucus group fungi because the dehydration process did not kill their spores and they were able to grow at low relative humidities. A second type of deterioration that appears on dried prunes is caused by the decay of fresh prunes by Monilinia fructicola or M. laxa. This type of decay is usually initiated while the fruit is still on the tree and is stopped by the heat of dehydration. A characteristic sign of this disorder is the presence of sporodochia of these fungi on the dried fruit. The third type of deterioration is a condition of unknown cause that is referred to by the prune industry as "box rot" (Fig. 1). The main symptoms of this condition are macerated areas on the dried fruit which are soft and sticky, and have a fruit skin which sometimes tends to slip with slight pressure. The purpose of this study was to find the cause of this disorder (herein termed "deterioration") of dried prunes.

MATERIALS AND METHODS

Inoculation study. French prunes from the experimental farm of the University of California, Davis were placed in sterilized net bags (50 per bag), surface-sterilized for 3 min with 0.525% sodium hypochlorite, air dried, dipped in a fungal spore suspension consisting of at least 5000 spores/ml, placed in large plastic bags and incubated at 28 C. The fungi used for individual inoculations were Alternaria alternata (Fr.) Keissler, Aspergillus chevalieri (Mangin) Thom and Church, A. japonicus Saito, Botrytis cinerea Pers. ex Fr., Cladosporium herbarum (Pers.) Link ex S.F. Gray, Monilinia fructicola (Wint.) Honey, M. laxa (Aderh. & Ruhl.) Honey, Mucor circinelloides V. Tiegh, Penicillium expansum Link ex Thom, Rhizopus arrhizus Fischer ATCC # 11145, R. arrhizus isolate # 2, R. arrhizus isolate # 3, and R. stolonifer (Ehrenberg ex. Rr.) Lind. Each inoculation treatment consisted of 48 net bags with the prunes in one-half of the bags injured by stabbing approximately ~~one-quarter of an inch~~ 0.5 cm deep with a dissecting needle. Thus there were 24 bags of injured and 24 bags of noninjured prunes; 6 bags of each were immediately placed in cold storage (0 C) and another 6 bags of each after 24, 48 and 68 hr of incubation at 28 C. The injured prunes were kept separated from the noninjured prunes within the dark incubation chamber. Prunes held at 28 C were examined daily for decay when the 24-, 48- and 68-hr samples were removed. They were considered decayed if the skin of the fresh fruit was macerated. Samples were left at 0 C for 1 to 3 days before they were commercially dried. After drying, the 300 prunes from the 6 bags were bulked into a single plastic bag. Subsamples were

time of bin filling with a spore suspension of Rhizopus arrhizus # 2 plus R. stolonifer were placed in each section of the bin, in order to determine if Rhizopus spp. would cause deterioration of noninoculated fruit. The inoculated fruit was placed approximately 10 cm below the upper fruit surface in each section of the bin; likewise, 25 punctured fresh prunes were inoculated with a spore suspension of Monilinia laxa plus M. fructicola for the same reason, and placed in each section. The inoculated fruit was placed close to the edge of the sections and the middle of each section was considered the noninoculated control. Noninoculated fruit, adjacent to those inoculated, in each section of each bin were sampled after 24, 48 and 72 hr. Nine hundred prunes, comprising 300 from each side and 300 from the middle of each section were sampled. They were examined for visible mold, placed in sterilized net bags (50 per bag) and dried commercially. Once dried the fruit was bulked into 300-prune samples representing the location of each section, and random samples were taken for evaluation of deterioration (50 prunes).

Enzyme study. Pectinase was obtained from fresh prunes by surface-sterilizing ripe prunes for 3 min with 0.525% sodium hypochlorite and placing in 0.5-1 jars after injuring with a 0.6-cm-dia. glass rod. The 0.5-1 jars were sealed with filter paper to insure proper aeration and placed in plastic bags after the fruit was inoculated with a 10-ml spore suspension of the test fungus. The spore suspension was poured over the prunes and distributed by rotating the jar to insure contact with all the fruit. The fungi were allowed to grow on the prunes for 60 hr at 28 C before the juice was collected by squeezing the fruit lightly and straining through cheesecloth. The Rhizopus fungi

drawn to test for deterioration (50 prunes), and pectinase (50 prunes). Deterioration was evaluated by taking the samples to prune-industry inspectors who specialize in scoring prune defects. They scored any damage to the prune skin with the exception of cracks as deterioration. Their definition was less precise than ours and could include a wider range of causes. The test for pectinase is described below.

Field study. Three standard prune harvest bins, each divided into 4 sections by wooden partitions, were filled to three-quarters capacity with fresh prunes from three different fruit growing areas of California (Fig. 2). Fruit from the Tulare area (southern San Joaquin Valley) was harvested at 12:30 PM, Aug. 27, 1980 by shaking 3 trees and conveying the fruit directly into the bin; fruit from the Yuba City area (central Sacramento Valley) was obtained similarly at 9:30 AM on Aug. 29, 1980 by shaking 2 trees; and fruit from a coastal area near Healdsburg was harvested by knocking the fruit on bare ground first, picking it up 3 hr later, and placing it in the bin at 11:00 AM, Aug. 30, 1980.

Fruit maturity at harvest, as measured by the fruit-flesh firmness, averaged 1.27 kg on the pressure gauge for Healdsburg, 2.63 for Tulare and 2.77 for Yuba City. These bins were immediately brought to the experimental farm of the University of California, Davis and held in the open air exposed to direct sun for 3 d. Fruit temperature a short distance below the upper fruit layer (Fig. 3) was continuously recorded in both the Tulare and healdsburg bins with model 2200 Marshalltown recorders (Marshalltown Manufacturing Co., Marshalltown, IA).

Four net bags, each containing 25 fresh prunes that had been punctured by a dissecting needle 0.5 cm deep and inoculated at the

were also inoculated on diced healthy prunes which had been autoclaved for 15 min to be absolutely certain of the enzyme source. Pectinase was obtained from dried prunes by soaking the fruit for 16 hr in 0.01M phosphate buffer (pH 7.2) with occassional stirring. The juice from infected fresh prunes and autoclaved prunes and the crude extract obtained from the dried samples were assayed for pectinase by the following procedure adapted from Strand et al (18). The liquid was filtered through a series of filters up to a 1.2 µm millipore filter and dialyzed against 0.01M acetate buffer (pH 4.5) with 4 changes at least 5 hr apart.

Pectinase activity was measured initially by the cup-plate test and if any activity was detected, by viscometry. The cup-plate method was adapted from Dingle et al (3). One percent sodium polypectate (Sunkist product No. 6024), 0.5% ammonium oxalate and 1.5% agar were blended into 150 ml of 0.1M acetate buffer (pH 5.0). After melting the agar and pouring the mixture into a suitable container the plates were inoculated by placing 50 µl of crude enzyme into a well made with a #3 corkborer and incubating at 39 C for 16 hr. The plates were developed by flooding with 5 N hydrochloric acid until formation of a white precipitate. The acid was then washed off and the diameter of the precipitated halo measured. Size 300 Cannon-Fenske viscometers were used. The substrate consisted of 4 ml of 2.1% sodium polypectate (Sunkist product No. 6024) and 1 ml of 0.6 M acetate buffer (pH 5.0). One ml of enzyme plus 5 ml of substrate were placed in viscometers immersed in a 30-C water bath. All enzyme analyses were done in triplicate. Sodium azide (0.02% w/v) was used in all buffers and assay reactions to prevent microbial growth. Activity was measured as the initial, approximately linear decrease in viscosity, in centipoise per min.

Fresh prunes were vacuum infiltrated with a solution containing 1 mg/ml of pectinase (Polygalacturonase, E.C. No. 3.2.1.15 from the Sigma Chemical Co. St. Louis, MO 63178). The prunes than were dried and tested for the slip skin condition by applying a slight pressure to the dried prune skin.

The effect of heat on pectinase from fresh fruit infected with Rhizopus was compared to pectinase from diseased dry fruit. The various enzymes were heated for 10 min at temperatures of 40, 50, 60, 70, 80, 90 and 100 C, cooled by plunging in cracked ice and stored at 0 C for viscometric analysis.

Characterization of pectinase from diseased and Rhizopus infected dried prunes. The pH optimum was determined by modifying the viscometric method. One percent w/v citrus pectin (Sunkist product No. 3467) was used for the substrate with the following buffers; 0.05M citrate-phosphate (3.0, 4.0, 5.0 and 6.0 pH), 0.05 M phosphate (7.0 pH) and tris (hydroxymethyl) aminomethane (8.0 and 9.0 pH). The effect of Ca^{++} was also studied by adding 0.001 M Calcium chloride per 100 ml to each of the buffers and measuring activity.

The thiobarbituric acid test (21) was used to detect the presense of unsaturated galacturonic acid and hence lyase activity. The reaction mixture consisted of 2 ml of 1.0% (w/v) citrus pectin (Sunkist product No. 3467), 0.5 ml of 0.05M citrate-phosphate buffer (pH 5.0) containing 0.001 M calcium chloride in 100 ml and 0.5 ml of crude enzyme. The reaction was allowed to occur for 24 hr. In 0.2 ml of reaction mixture 0.25 ml of 0.025N potassium iodate in 0.125 N sulfuric acid was added. After 20 min at room temperature 0.5 ml of 2% (w/v) sodium arsenite in

0.5 N hydrochloric acid was added with shaking and allowed to stand for 2 min. Two ml of 0.3% (w/v) 2-thiobarbituric acid (pH 2.0) was added with stirring and the mixture was heated at 100 C for 10 min. After cooling the optical density was measured at 548 um in a spectrophotometer.

The macerating ability of the enzymes were compared with and without the calcium ion present (7). Flasks (125 ml) containing 25 ml of 0.1 M sodium acetate buffer (pH 5.1) or 0.1 M calcium acetate buffer (pH 5.1), 20 ml of distilled water and 5 ml of enzyme were used. Seven g of ripe prunes cut into 5 mm cubes were added to each flask. The flasks were shaken for 7 hr at 210 rev/min in a 28 C room. Free cells were determined by sieving through a 0.83 mm (20 mesh) sieve, and 10 ml was placed in a graduated test-tube. The volume (ml) of single cells was measured when the cells had sedimented to an unchanged level.

Source of inoculum for Rhizopus infection. A selective medium (14) was used to determine if Rhizopus spores were present on prunes at harvest time. Ten fruit per tree were collected from 3 trees in each of 3 orchards. The 10 prunes were washed for 1 min with sterile distilled water which was subsequently diluted 1:10. Ten µl of this wash was distributed over the surface of a std. petri plate containing Rhizopus selective medium modified to contain one-tenth the prescribed amount of crystal violet. The plates were read after 2d for colonies of Rhizopus. Similarily 1g of soil from each of the orchards was placed in 10 ml of sterile water, diluted 10:1 and 10 µl was spread on the selective medium.

RESULTS

Inoculation study. After 24 hr, Rhizopus arrhizus #3 decayed 5% of the fresh prunes, while the other genera of fungi tested did not cause decay (Table 1). When this fruit was dried 14% of the R. arrhizus #3 and 2% of the R. stolonifer-infected fruit were deteriorated. None of the other fungi tested caused deterioration. After 48 hr both species of Rhizopus caused considerable decay. At this time A. japonicus and Penicillium expansum began to produce some decay. When the prunes were dried the deterioration of the R. arrhizus #3 fruit had increased from 14% to 35% and that of R. stolonifer fruit had increased from 2 to 25%. Monilinia fructicola caused 12% of the fruit to be deteriorated and the rest of the fungi caused a lesser degree of deterioration. After 68 hr, up to 75% of the fruit showed Rhizopus decay. In all cases where a high percentage of the fruit was decayed, Rhizopus mycelium was observed. After the fruit was dried Rhizopus arrhizus #3 was shown to have deteriorated 94% and R. stolonifer 74% of the fruit sampled. The 40% deterioration in the A. japonicus sample was in a large part due to Rhizopus contamination since 16.7% of the fresh fruit was covered with Rhizopus mycelium; whereas in treatments not contaminated with Rhizopus only a small percentage of the dried fruit was deteriorated.

Fruit that was surface-sterilized but not inoculated remained free of decay, and deterioration was only 2.0% even after 68 hr of incubation at 28 C (Table 1). On the other hand, 10% of the fruit naturally contaminated with Rhizopus were deteriorated in 24 hr and 58% in 68 hr.

R. arrhizus #3 deteriorated a higher percentage of fruit in

24 hr that was deliberately injured than those not injured (Fig. 4). However deliberate injury did not appear to be important in causing higher rates of deterioration after 48 and 68 hr. For example less fruit infected by R. arrhizus #3 that had been deliberately injured were deteriorated after 68 hr than those not injured.

Field study. Fresh prunes from the three different fruit growing areas of California were examined for decay at the time the bins were filled and inspected when they were dried for deterioration. Those from Tulare and Yuba City had 8.2% and 3.8% deterioration, respectively, while the Healdsburg fruit did not contain any initial deterioration (Table 2). Natural infection by Monilinia was present on some of the fruit harvested in Tulare, and part of the initial 8.2% deterioration could be attributed to Monilinia decay. The 3.8% deteriorated fruit in the Yuba City sample was due to an unexplainable factor as we did not observe any organism growing on this fruit before it was dried.

After the fruit was held for 24 hr the prunes from the middle areas of the bins, where no inoculated fruit had been placed, were sampled, examined for decay, and dried. None of the Yuba City fruit was deteriorated after drying while 4% of that in the Healdsburg and Tulare bins were deteriorated. After 48 hr a large increase (17.6%) in deteriorated fruit occurred in the Healdsburg bin while the amount of deteriorated fruit in the Tulare (4.2%) and Yuba City (0.0%) bins remained at a very low level. Rhizopus mycelium was observed on the fresh fruit sampled from this control section of the Healdsburg bin. After 72 hr all 3 bins showed a large increase in deteriorated dried fruit. Rhizopus mycelium

was observed on fresh fruit sampled from the control section in all three cases.

After 24 hr, the Monilinia inoculated area of each bin contained slightly more fruit that was graded deteriorated after drying than the noninoculated middle control area. After 48 hr there was a small increase in deteriorated fruit from this area of each bin. After 72 hr large increases in the number of deteriorated prunes sampled from the Tulare (24.0%) and Healdsburg (30.0%) bins occurred in the Monilinia areas of these bins. Before drying, Rhizopus mycelium was observed in these samples but not in the Yuba City sample, where only 7.8% of the fruit was deteriorated.

After 24 hr, fruit from the Rhizopus inoculated area of each bin that was graded deteriorated after drying varied little from that of the control in all three cases. After 48 hr large increases in the amount of deteriorated fruit occurred in all 3 bins. Rhizopus mycelium had been observed before drying on samples from the Tulare and Healdsburg bins and this observation coincided with large amounts of deteriorated dried fruit. After 72 hr, in all 3 cases, the samples of fresh fruit were infected with Rhizopus and when dried both the Yuba City and Healdsburg samples contained the highest amount of deteriorated prunes, 30% and 75% respectively. The Tulare bin contained more fruit that were graded deteriorated after 48 hr than after 72 hr. This difference can probably be attributed to sampling method. Where small numbers of Rhizopus inoculated fruit were placed in each of the 3 bins and held either 48 or 72 hr before drying, significantly higher numbers of adjacent noninoculated fruit became infected and, consequently, larger numbers of dried fruit were found to be deteriorated.

Inoculum for Rhizopus infection. Fruit was found to be contaminated with Rhizopus spores at the time of harvest (Table 3). Rhizopus was present on the fruit hanging in the trees and also in the soil.

Enzyme study. Pectinase activity was measured for fungi that were suspected to cause prune deterioration by assaying the crude enzyme produced on fresh prunes which had been inoculated and incubated at 28 C for 60 hr (Table 4). Aspergillus japonicus was the most prodigious elicitor of pectinase. The two species of Rhizopus studied were also good producers of pectinase, with Rhizopus arrhizus #2 producing almost as much activity as A. japonicus. R. stolonifer produced less enzyme activity than R. arrhizus but about equal to that of Penicillium expansum.

Absorption of pectinase purchased from the Sigma Chemical company into fresh prunes by vacuum infiltration produced deteriorated fruit upon drying. Thirty-four of 70 prunes exhibited a slip-skin condition whereas none of the prunes infiltrated with pure water showed this symptom.

Deteriorated prunes sampled at various times from a central processing plant fluctuated in the amount of pectinase detected (Table 5). The viscometry and cup-plate tests indicated that 4 of the 6 deteriorated samples had pectinase activity, while 2 had no significant pectinase activity.

Prunes inoculated with Rhizopus and dried displayed pectinase activity (Fig. 4). Deteriorated samples 3 and 4 had activities similar to those in the R. arrhizus-infected fruit, and deteriorated samples 1 and 2 were similar to the R. stolonifer-infected fruit.

Subjecting Rhizopus arrhizus crude enzyme to temperatures from 40 to 100 C resulted in a heat inactivation curve similar to that

found in a preparation from one of the naturally-deteriorated samples (Fig. 5). R. stolonifer crude enzyme lost nearly all its activity after being heated to only 50 C.

Characterization of pectinase from naturally-deteriorated and Rhizopus-deteriorated dried prunes. The pH optimum curves for both Rhizopus-deteriorated and naturally-deteriorated prunes were similar (Fig. 6). The addition of calcium chloride stimulated both enzymes and the pH optimum for both without calcium chloride was pH 5.0.

The results of the thiobarbituric acid test were negative. The enzyme from Rhizopus-infected deteriorated dried prunes or that from naturally-deteriorated dried prunes did not cause any increase in absorbance, thus indicating that no unsaturated galacturonic acid was being produced.

The maceration test (Table 6) showed that both the enzyme from naturally-deteriorated prunes and Rhizopus-deteriorated prunes caused maceration at pH 5.1 in the presence of sodium acetate buffer. Neither enzyme was effective when calcium acetate was used as the buffer at the same pH.

DISCUSSION

Evidence was provided that links deterioration of dried prunes to infection by Rhizopus spp. We have shown how the condition can be reproduced within 24 hr by inoculating fresh prunes with Rhizopus spp. and drying them in the normal manner (Table 1). Field studies indicate that the fungus will quickly spread through a bin of fresh fruit if left for 48 hr under normal prune harvest temperature conditions. In a limited survey we found

inoculum both on prunes hanging in the tree and in the orchard soil. Furthermore we found similar pectinase activity in a crude enzyme extract from commercially-deteriorated as well as in a similar extract from Rhizopus-deteriorated dried prunes. Characterization of these enzymes showed them to have the same pH optimum, the same reaction to calcium and the same heat inactivation temperature.

Some questions exist between some of the enzyme samples from natural sources and that from Rhizopus-deteriorated prunes. For example, deteriorated fruit obtained from commercial sources and that produced by inoculating prunes with Rhizopus spp. and drying do not always contain appreciable enzyme (Fig. 4). We found that R. stolonifer which caused 74% of the dried fruit to be deteriorated was so low in pectinase that the activity was barely measurable. Apparently, most of the pectinase is destroyed by dehydration which explains why it is not detected in all samples. Deteriorated sample #6 had an exceptionally high pectinase activity, indicating that some of the fruit in this sample may have been decayed by Aspergillus spp. and/or Penicillium spp. as well as Rhizopus spp.

Live spores of Aspergillus glaucus and A. japonicus are often found on prunes after drying (5). When we inoculated fruit with A. chevalieri, the most common fungus on dried prunes (4), we detected no decay of the fresh prunes and deterioration of only high-moisture-content, dried prunes (≥25%) after several days of storage (16). This decay was always accompanied by green sporulation or yellow cleistothecia characteristic of A. glaucus. A. japonicus did cause some deterioration of the fresh prunes and produced a very active pectinase. It may be involved in some deterioration if fruit is held at high temperature 3 or more days before drying. This fungus is not the primary

cause of deterioration because of the type of rot it produces and the long period it takes to cause damage.

Monilinia spp. have been suggested as the chief causal agents of the condition under investigation (2). We were unable to produce the typical symptoms of this deterioration quick enough and severe enough with these fungi to support this contention. Perhaps when Monilinia spp. occurred in combination with Rhizopus spp. the two were confused and the deterioration was attributed to the Monilinia spp. Generally, deterioration caused by Monilinia spp. can be distinguished from that caused by Rhizopus spp. because Monilinia spp. produce sporodochia on the fruit.

The amount of fruit deteriorated by Rhizopus in a bin depends on the species of fungus, temperature of the fruit, amount of injured fruit, fruit maturity, humidity of the air surrounding the fruit and the length of time the fungus grows on the fruit before dehydration. Rhizopus spp. can be divided into two categories based on the highest temperature at which they can grow. We found that the high temperature R. arrhizus produced more pectinase than the more common low temperature R. stolonifer (Table 5). This appears to coincide with an earlier and more extensive deterioration by R. arrhizus (Table 1, Fig. 4). Temperature of the fruit in the bin is important in determining if Rhizopus decay will occur and how fast it will progress. On potato dextrose agar, mycelium of R. stolonifer grows optimally at 25 C with a maximum at 32.2 C and minimum at 4.4 C (13) whereas R. arrhizus grows optimally at 35-36 C (22). Fruit injury is important since Rhizopus infection initially occurs only on injured tissue (23). Our experiments to demonstrate that injury causes increased deterioration were inconclusive (Fig. 4). Apparently

there was enough natural injury of the fruit so this was not a factor. R. stolonifer requires a minimum relative humidity of 92-94% for development with the optimum being 98% when grown under controlled laboratory conditions on nutritive media (12). High relative humidities are almost assurred in a bin of mature respiring fresh fruit. The fungus grows out from single-infection foci releasing enzymes which act to soften the skin of the infected fruit. The mycelium arising from an infected fruit or from a sporangiospore can penetrate healthy fruit in contact with infected fruit in the absence of visible wounds (1). The longer infection is allowed to proceed the greater will be the amount of fruit deterioration. Our experiments show that deterioration increased over time when the fruit was inoculated with Rhizopus spp. (Table 1) and when bins containing Rhizopus inoculated and noninoculated fruit were held at high temperature 48-72 hr before the fruit was dried (Table 2).

Liu and Luh (8) showed that when R. arrhizus was grown on ripe apricots one of the pectinase enzymes produced was endo-polygalacturonase. Similarly our experiments indicated that the pectinase enzyme in deteriorated prunes was a polygalacturonase because it had an optimum pH of 5.0 and did not release unsaturated galacturonic acid. Luh et al (9) found that the crude enzyme from apricots rotted by R. arrhizus was heat resistant. When we compared the activity (-0.0083 centipoise/min)of the pectinase from our dried prune sample deteriorated by R. arrhizus with that produced on fresh prunes (-0.1141 centipoise/min) decayed by the same fungus we found that 7.2% of the original activity was retained indicating heat resistance. We did not observe any pronounced bimodal heat stability curves (Fig. 5) for R. arrhizus

or R. stolonifer as reported by Strand et al (18). This could be because our crude enzyme was not in 30% (w/v) sucrose as used by these reseachers.

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Table 1. Correlation between the percent decay of fresh prunes inoculated with various fungi and incubated at 28 C for 24, 48 and 68 hr and the percent deterioration of the dried fruit

Fungus	24 hr		48 hr		68 hr	
	Decayed[a]	Deteriorated[b]	Decayed	Deteriorated	Decayed	Deteriorated
Rhizopus arrhizus #3	5.0	14.0	25.0	35.0	50.0	94.0
R. stolonifer	0.0	2.0	7.5	25.0	75.0	74.0
R. arrhizus #2	0.3	0.0	4.0	14.0	35.0	48.0
Aspergillus japonicus	0.0	0.0	2.0	8.0	33.3[c]	40.0
R. arrhizus #1	0.0	0.0	3.0	4.0	40.0	38.0
Cladosporium herbarum	0.0	0.0	0.0	2.0	13.3[c]	10.0
Monilinia laxa	0.0	0.0	0.3	0.0	3.3	10.0
Penicillium expansum	0.0	0.0	3.3	4.0	5.7[c]	10.0
Mucor circinelloides	0.0	0.0	0.0	4.0	1.7	6.0
M. fructicola	0.0	0.0	0.0	12.0	4.0	6.0
Botrytis cinerea	0.0	0.0	0.0	2.0	0.3	4.0
A. chevalieri	0.0	0.0	0.0	0.0	1.7	4.0
Alternaria alternata	0.0	0.0	0.0	4.0	3.3[c]	2.0
Noninoculated #1[d]	0.0	0.0	0.0	0.0	0.0	2.0
Noninoculated #2[e]	3.3[c]	10.0	10.0	12.0	17.7	58.0

[a]Number of fruit decayed were estimated by counting the number of fruit with broken skins.

[b]Number of fruit deteriorated were rated by industry inspectors from a sample of 50 dried prunes.

[c]This fruit was contaminated with visible Rhizopus mycelium.

[d]Noninoculated #1 prunes were surface-sterilized before incubation.

[e]Noninoculated #2 prunes were incubated without surface-sterilization.

Table 2. Percent deterioration of noninoculated dried prunes harvested from 3 geographic areas held in the same bin with *Monilinia*-and *Rhizopus*-inoculated fresh prunes

Incubation period before drying (hr)	Tulare			Yuba City			Healdsburg		
	C[a]	M[b]	R[c]	C	M	R	C	M	R
0	8.2[d]	-	-	3.8	-	-	0.0	-	-
24	4.0	6.0	10.0	0.0	2.0	0.0	4.0	6.2	1.9
48	4.2	12.0	34.0[e]	0.0	5.9	18.0	17.6[e]	9.8	38.5[e]
72	28.8[e]	24.0[e]	24.5[e]	11.8[e]	7.8	30.0[e]	28.0[e]	30.0[e]	75.0[e]

[a]C=Control. Each bin section (Fig.2) was divided into 3 parts with the middle, noninoculated prunes designated as control.

[b]M=*Monilinia* spp. Twenty-five freshly harvested ripe prunes were inoculated with *M. fructicola* and *M. laxa* and placed on one side of each section.

[c]R=*Rhizopus* spp. Similar to the preceding except *R.arrhizus* and *R. stolonifer* were used as inoculum.

[d]This initial deterioration was likely due to brown rot since the fruit was naturally infected with *Monilinia*.

[e]*Rhizopus* mycelium was observed the fresh prunes.

Table 3. Occurrence of *Rhizopus* spp. on prunes and in prune orchards

Orchard	Number of *Rhizopus* colonies	
	Fruit wash	soil
Tulare #1	27	3
Tulare #2	7	2
Healdsburg	1	2
Yuba City	14	16

Table 4. Pectinase activity of crude enzyme from fresh prunes inoculated with specific fungi and incubated for 60 hr at 28 C

Fungus	Pectinase activity	
	cup-plate[a] (mm)	viscometry[b] (centipoise/min)
Noninoculated	0	-0.0015 V
Monilinia fructicola	2	-0.0019 V
M. laxa	3	-0.0035 V
Penicillium expansum	9	-0.0392 W
Rhizopus stolonifer	7	-0.0401 W
R. arrhizus #3	12	-0.1141 X
R. arrhizus #2	13	-0.1466 Y
Aspergillus japonicus	15	-0.2364 Z

[a]Diameter of cup subtracted from diameter of cup plus pectinase induced halo.

[b]Vaues in the viscometry column not followed by the same letter are significantly different (P=0.05).

Table 5. Pectinase activity of crude enzyme from commercially-deteriorated dried fruit

Sample	Pectinase activity	
	Cup-plate[a] (mm)	Viscometry[b] (Centipoise/min)
Healthy[c]	0	-0.0013 V
Deteriorated #1	0	-0.0012 V
Deteriorated #2	2	-0.0024 V
Deteriorated #3	3	-0.0070 W
Deteriorated #4	5	-0.0158 X
Deteriorated #5	8	-0.0226 Y
Deteriorated #6	9	-0.0479 Z

[a]Diameter of cup subtracted from diameter of cup plus pectinase induced halo.

[b]Values in the viscometry column not followed by the same letter are significantly different (P=0.01).

[c]Dried fruit free of visible defects.

Table 6. Effect of calcium on the macerating ability of two enzymes as measured by the release of free cells (ml) in 7hr of shaking

Enzyme	Buffer (0.1M pH 5.1)	
	Sodium acetate	Calcium acetate
Water	1.0	1.1
Deteriorated #5	1.9	1.2
Rhizopus arrhizus #2	2.4	0.7

Fig. 1. Prunes from commercial sources showing signs of "box rot" deterioration. The affected areas are indicated by arrows.

Fig. 2. Partition of a harvest bin for incubating prunes from Tulare, Yuba City, and Healdsburg for various periods at ambient temperatures. The incubation periods and location of noninoculated prunes (C) or prunes inoculated with Monilinia (M) or Rhizopus (R) are shown. At the end of the incubation periods noninoculated fruit from these three areas were removed for drying and subsequent assessment of deterioration.

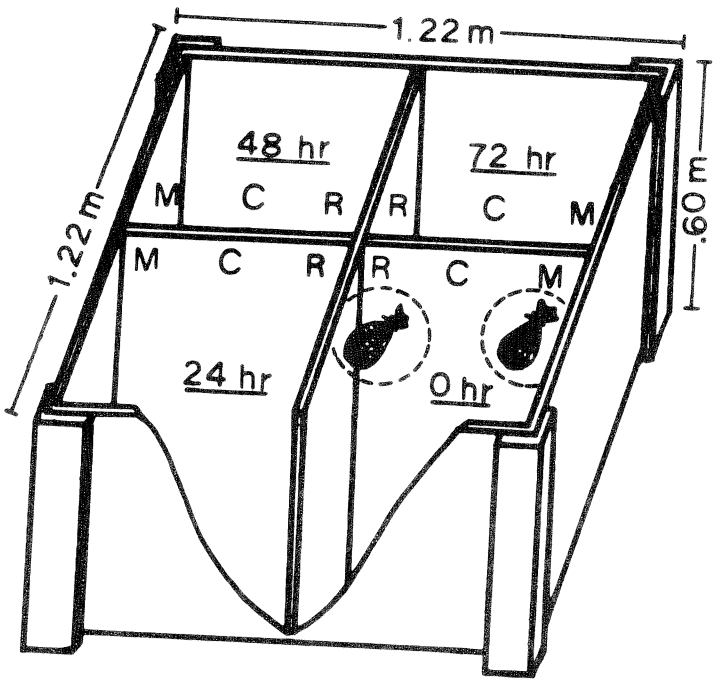


Fig. 3. Temperature of fruit near the top of the Tulare bin (starting at 12:00 PM, Aug. 27, 1980) and the Healdsburg bin (starting at 12:00 PM, Aug. 30, 1980) left at ambient temperature in the direct sun for over 3 d.

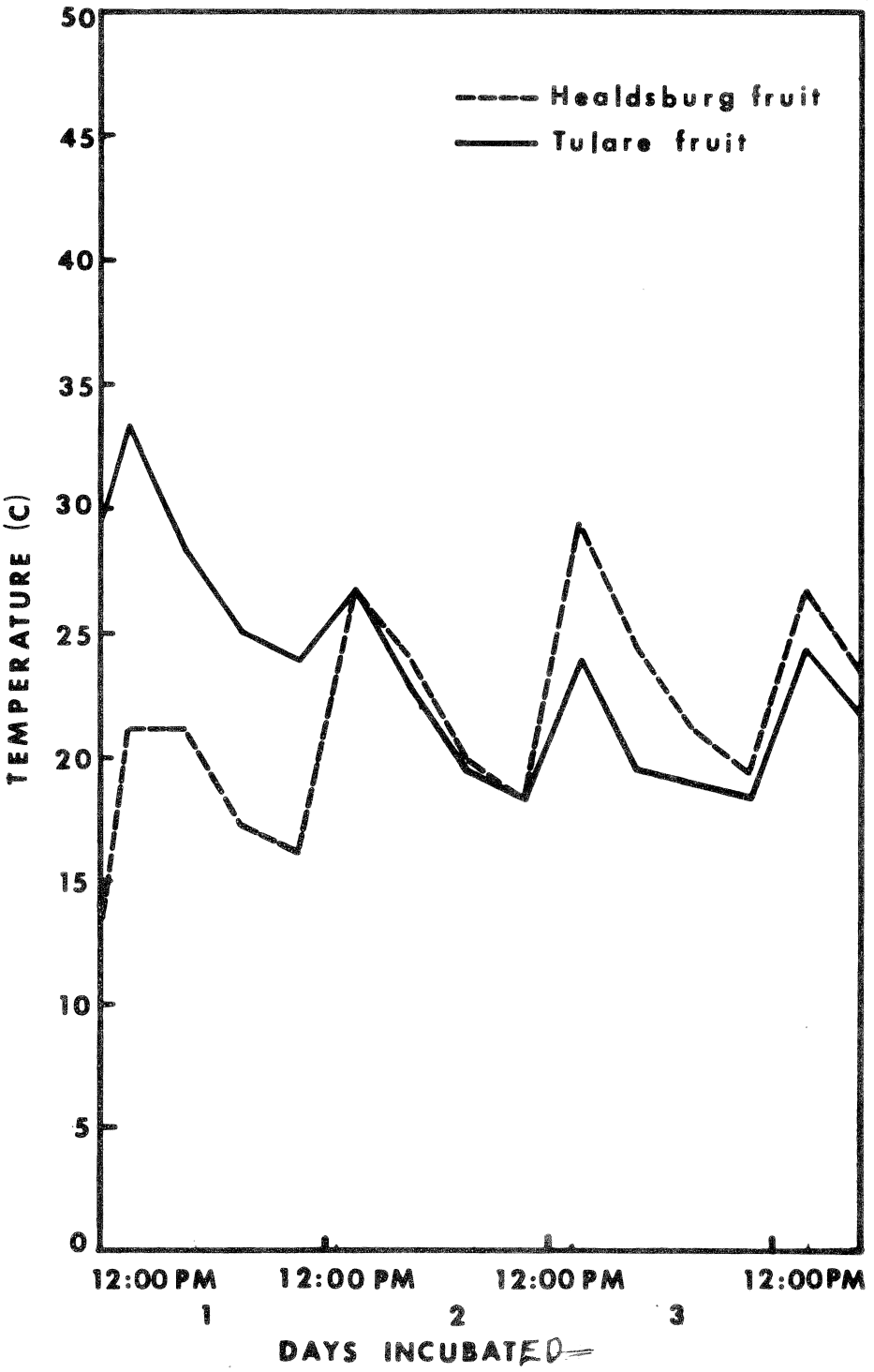


Fig. 4. Deterioration of dried, injured and noninjured prunes that had been inoculated with Rhizopus spp. and incubated 24, 48, and 68 hr at 28 C before drying. Pectinase activity of fruit inoculated with R. arrhizus after 48 hr incubation and of fruit inoculated with R. arrhizus and R. stolonifer after 68 hr incubation. The pectinase activity of 6 commercially deteriorated fruit samples is also shown.

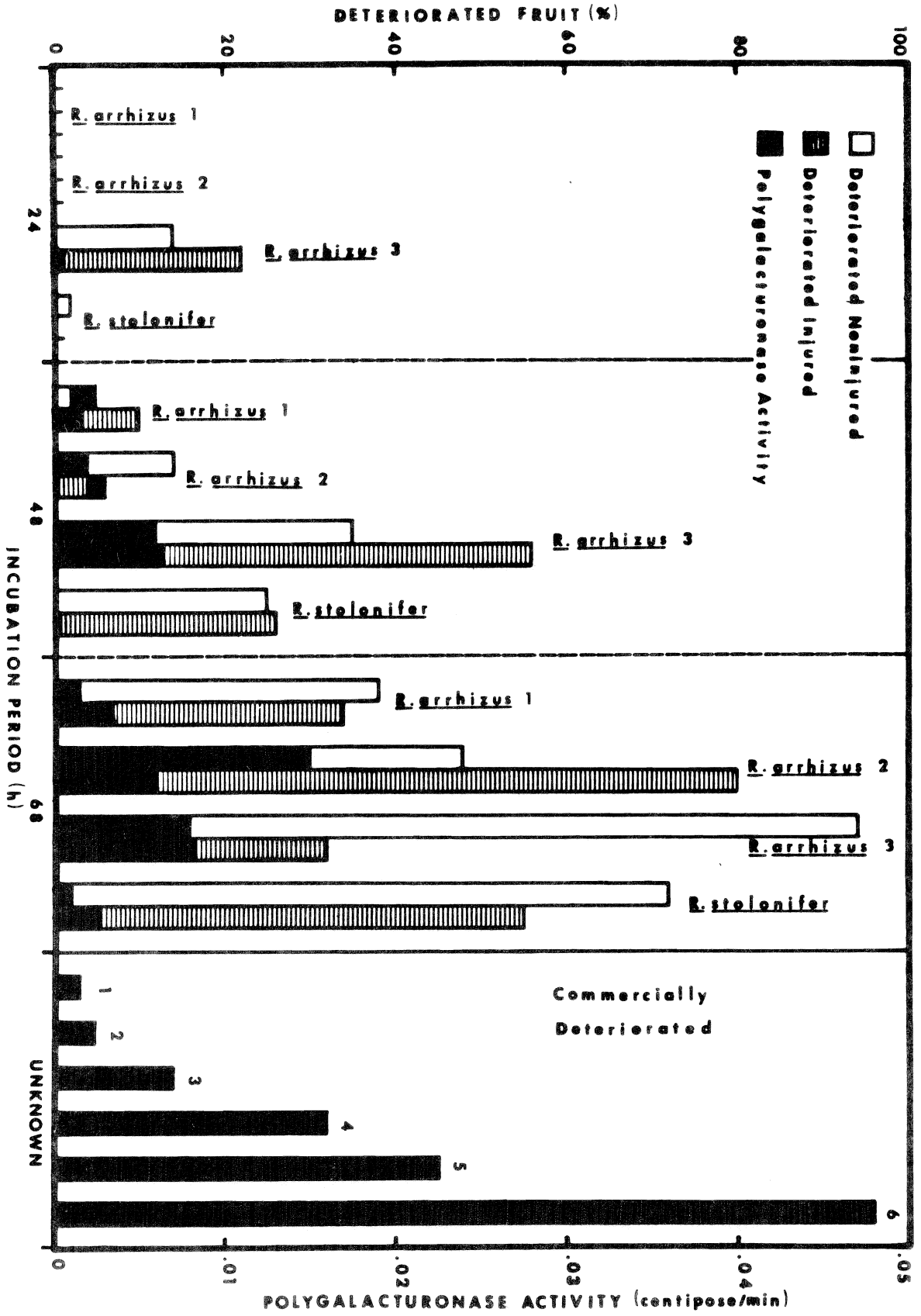

DETERIORATED FRUIT (%)
0
20
40
60
80
100
R. arrhizus 1
R. arrhizus 2
R. arrhizus 3
R. stolonifer
Polygalacturonase Activity
Deteriorated Injured
Deteriorated Noninjured
24
48
68
INCUBATION PERIOD (h)
R. arrhizus 1
R. arrhizus 2
R. arrhizus 3
R. stolonifer
R. arrhizus 1
R. arrhizus 2
R. arrhizus 3
R. stolonifer
Commercially
Deteriorated
UNKNOWN
1
2
3
4
5
6
0
.01
.02
.03
.04
.05
POLYGALACTURONASE ACTIVITY (centipose/min)

Fig. 5. Effect of heating (10 min at 40-100 C) pectinase produced by R. arrhizus #2 and R. stolonifer on fresh prunes, and on that found in commercially deteriorated sample #5 (Table 6).

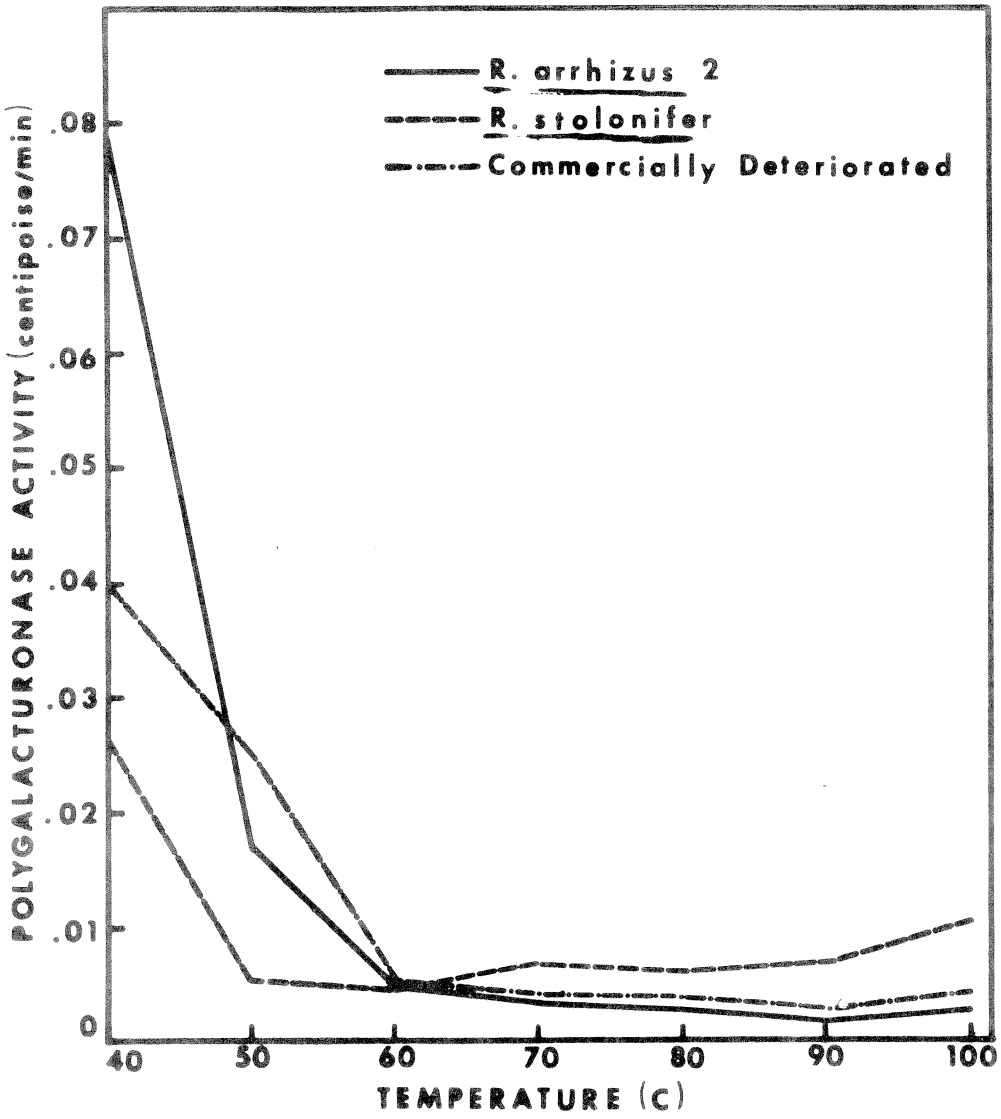
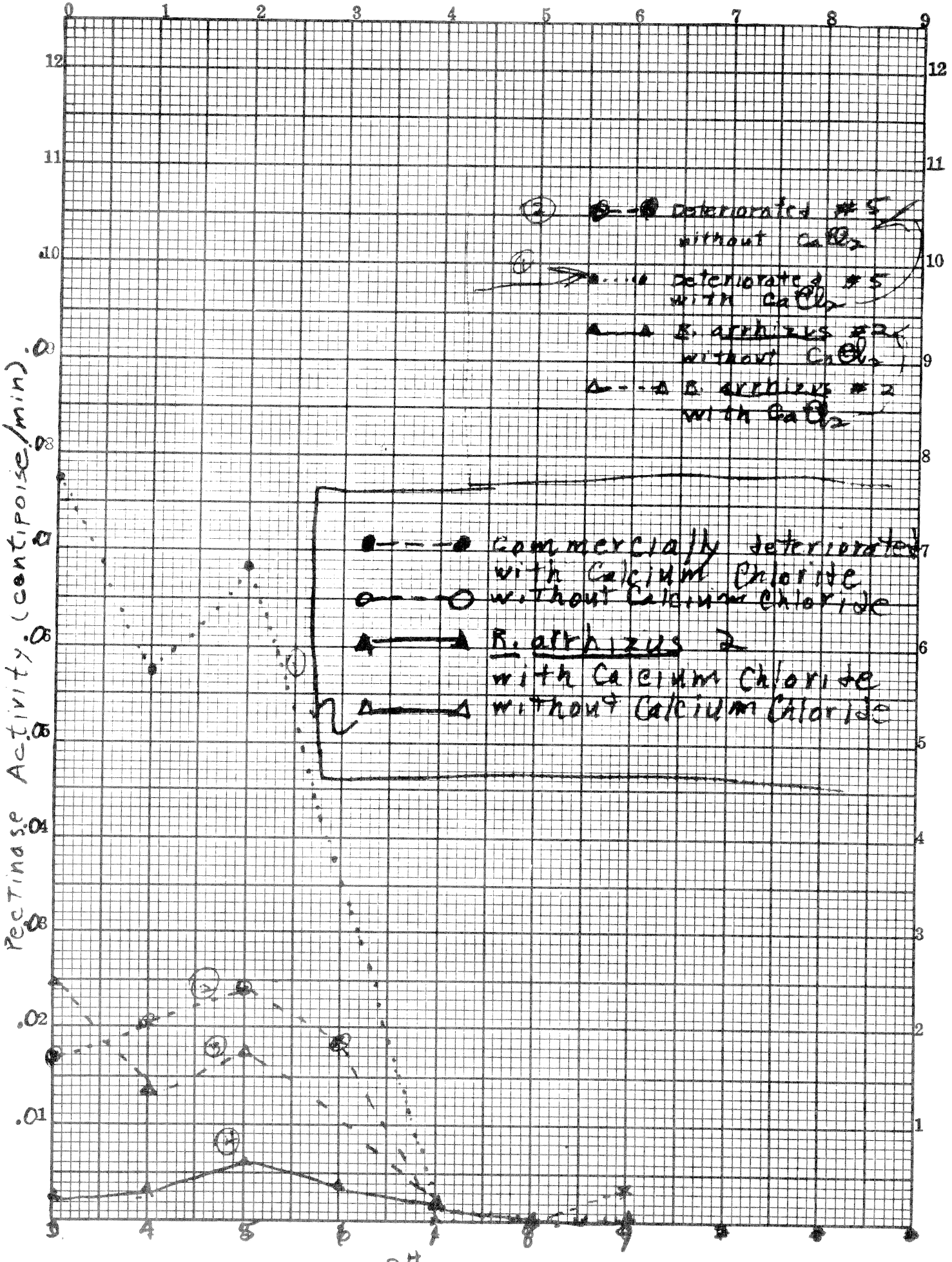


Fig. 6. pH curve and the effect of calcium chloride on crude pectinase enzyme from naturally-deteriorated #5 and R. arrhizus #2 deteriorated dried prunes.


Pectinase Activity (centipoise/min)
pH
Deteriorated #5 without CaCl2
Deteriorated #5 with CaCl2
R. arrhizus #2 without CaCl2
R. arrhizus #2 with CaCl2
Commercially deteriorated with Calcium Chloride
without Calcium Chloride
R. arrhizus #2 with Calcium Chloride
without Calcium Chloride