2-AMINO-PYRIDINE, A PROMISING INHIBITOR OF DECAY IN ORANGES[1]

Introduction

Antiseptics have long been used for checking decay in citrus fruits. For this purpose they must possess high fungicidal properties, and at the same time be non-injurious to the commodity or to those who consume it over a long period. A number of chemicals that will give the desired degree of decay control are so toxic to animals that their use on foodstuffs is not permissible. Yet it is reasonable to assume that if the search is carried on long enough, someone will find an excellent decay inhibitor that meets all requirements of the health authorities.

With this objective in view, the U. S. Department of Agriculture Subtropical Fruit Field Station at Orlando, Florida, continued throughout the season of 1946-47 a search for an outstanding effective but safe material for treating citrus fruits to prevent the development of decay at any time during the marketing period. In the 1946-47 season several hundred compounds of established fungicidal or fungistatic properties, including a number of pyridine derivatives, were used in screening tests on oranges to determine their decay-inhibiting qualities. From this number several were outstanding for rot control, but the 2-amino-pyridine, hereinafter referred to as "2-AMP" for brevity, gave the most promising results.

Inasmuch as pyridine is a natural constituent of bone oil, there appeared some basis for suspecting that use of 2-AMP[2] might meet the requirement of being non-toxic. Concurrently with the execution of the tests reported herein, other agencies have initiated feeding experiments to ascertain whether the material could be applied to oranges without detriment to the consumers of treated fruit. These tests have not been completed, nor have the findings to date been released. Pending a determination of the toxicity of this material its use on fruit is not sanctioned. However, enough fruit-treating experiments have been executed to justify the release of a progress report on this phase of the study.

The first dipping tests made with this material at the Orlando laboratory were set up in early November, 1946. During the season more than 245 experimental lots totaling more than 13,000 fruits were used to evaluate 2-AMP as an inhibitor of decay in Florida oranges.

Experimental Procedure

These studies were confined almost exclusively to oranges that were ripe enough for market at the time of the testing under discussion. Such varieties as Parson Brown, Hamlin, Pineapple, Seedling, and Valencia, produced on mature trees, were used in their respective ripening seasons.

Upon delivery of the oranges to the laboratory from the groves, all lots of fruit were washed. While still wet they were transferred to a room maintained at 85°F. and 90 percent relative humidity. Here they were subjected to ethylene gas for 48 to 60 hours to accelerate the development of stem-end rot; however, the gassing treat-

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[2]The use of this material was suggested by the American Cyanamid Company, which supplied samples for screening tests.

ment renders fruit less liable to green mold rot.

The fruit was treated within a few hours after the gassing period. Some lots were immersed for a fixed period of 10 seconds in varying concentrations of 2-AMP in water or in wax emulsion diluted to proportions commonly applied to citrus. In other experiments with a constant concentration of the antiseptic, the temperature of the bath was varied from 80° to 125°F., and in still other tests the length of the exposure to the bath ranged from 10 seconds to 3 minutes. None of the treated lots were rinsed subsequently to remove the adhering chemical. The dipped fruit was placed in trays to dry in open air, then transferred to a holding room.

Inasmuch as 2-AMP is somewhat volatile, it seemed advisable to explore the possibilities of applying this material to fruit in much the same manner as diphenyl, a volatile fungistat, by means of treated wrapping tissues or case liners. The wraps were treated by immersing them in an alcoholic or water-wax-emulsion solution of 2-AMP, hung on lines to dry, and then stored for future use. The first test with treated wraps was made March 11, 1947. The fruit was wrapped and packed in the usual manner, then transferred immediately to the holding room.

Some inoculation experiments with green mold, Penicillium digitatum, were conducted on oranges harvested and inoculated the same day. The fruit was washed, pricked with ten needles to depths of about 1mm., and dipped in a rich suspension of fresh spores taken from rotting oranges. The inoculated lots were held in a near saturated atmosphere at 70°F. for varying lengths of time before treatment with the antiseptic in wax emulsion. After application of 2-AMP, the fruit was surface dried in moving air and promptly placed in crates lined with paraffin paper, to assure the maintenance of an environment of high humidity, and promptly placed in the holding room, which was continuously maintained at about 80 percent relative humidity and 70°, a temperature favorable to the development of the principal rots of Florida citrus fruits. The uninoculated lots were inspected weekly over a period of three weeks, long enough to cover most marketing operations, while the inoculated oranges were inspected at intervals of three days over a nine-day period.

Results

2-AMP in water or wax emulsion: The preliminary tests with 2-AMP, ranging in concentration from 10 to 2-1/2 percent, in water as well as in a wax emulsion commonly used on fruit as a fruit dip, were repeated several times with early and midseason oranges.

Inasmuch as all lots were subjected to ethylene gas, a treatment which is followed by less green mold than is ordinarily found in nongassed fruit, this rot developed in too small amounts even in the non-treated check lots to permit of safe interpretation. Therefore, only the data on stem-end rot (Phomopsis citri or Diplodia natalensis) will be considered in the initial tests.

Results reported in Table 1 show a remarkable control of stem-end rot by 2-AMP. In a water medium 10 percent 2-AMP gave the greatest control while 2 1/2 percent gave the least, and the difference in decay suppression between the 10 percent and 5 per cent solutions was not great. With fruit dipped in wax emulsion containing 2-AMP, the 2 1/2 percent solution was almost as effective as the 10 percent.

With these feeler tests on midseason oranges as a background, experiments were conducted with Valencia oranges immersed for 10 seconds in 5 and 10 pecent 2-AMP in water. Similar concentrations of the chemical were incorporated in the water phase of the wax emulsion, as another practical method of application for commercial operations. Table 2 gives the results from six groups of 11 tests lots each.

In the series treated with 2-AMP in water, the miscellaneous decay, mostly side rot, was not of sufficient magnitude to be

consequential. The greatest amount of green mold, 3.1 percent was found in the untreated check, and the least, 1.3 percent, in the fruit treated with 10 percent 2-AMP. However, stem-rot increased in the water-dipped checks from 6.9 percent in 7 days to 53.8 percent a week later, and to 70.2 percent after another week. The fruit dipped in 5 percent 2-AMP in water had 2.4 percent, 2.5 percent, and 3.8 percent stem-end rot, at the end of the first, second and third weeks respectively, whereas 10 percent solution of 2-AMP gave 1.3, 2.0 and 2.5 percent. Decay from all causes finally consumed 73.8 percent of the untreated fruit, and 5.8 and 4.2 percent in the lots receiving the weaker and stronger concentrations of 2-AMP respectively. When similar proportions of 2-AMP were added to the emulsion, the resultant decay control was of very nearly the same magnitude as with the water solution.

A consideration not evaluated in the table above is chemical injury to the rind of treated fruit. A trace of brown, slightly sunken spots, probably chemical injury, was observed in three of the eleven lots, following the application of 5 percent 2-AMP in water. The blemish increased measurably but probably not importantly, commercially speaking, during the holding period. No rind injury was observed on fruit receiving emulsion containing 5 percent 2-AMP. However, the 10 percent solutions in emulsions as well as in water caused an appreciable amount of rind injury which increased in number of affected fruits with the extension of the holding period, and which doubtless was present in sufficient proportions to be commercially significant. Rind injury developed more on the fruit treated with water solutions than on that treated with an emulsion containing the antiseptic.

2-AMP in water dip for 10 seconds, 1 minute, 3 minutes: In another series of experiments with gassed oranges, table 3, a 5 percent water solution of 2-AMP was applied to the fruits for 10 seconds, 1 minute, and 3 minutes, respectively, at a uniform concentration of 5 percent. In none of the lots did the miscellaneous wastage, mostly side rots, amount to as much as 2.0 percent, even after 3 weeks' holding. Likewise, green mold increased to only 2.4 percent in the check fruit, and to not more than 1.6 percent in the treated lots. In the case of stem-end rot, decay control was very effective with no significant differences due to the duration of the dip.

Not more than a trace of rind injury was

TABLE 1—TOTAL STEM-ROT IN GASSED EARLY AND MIDSEASON ORANGES DIPPED IN SEVERAL CONCENTRTATIONS OF 2-AMINO-PYRIDINE — HELD 6 WEEKS AT 70° F.

Treatment	No. Tests	No. Fruit	Stem-end Rot
			Percent
Check — water	3	75	57.3
10% 2-amino-pyridine in water	3	75	1.3
Check — water	9	242	65.3
5% 2-amino-pyridine in water	9	241	4.1
2 1/2% do	9	244	9.4
Check — emulsion	4	100	47.0
10% 2-amino-pyridine in water	4	100	4.0
5% do	4	100	3.0
2 1/2% do	4	100	5.0

TABLE 2—DECAY IN GASSED ORANGES TREATED WITH 2-AMINO-PYRIDINE (10 SECOND DIP, NO SUBSEQUENT RINSING)

Orlando, Florida—Spring, 1947

Treatment	No. Tests	No. Fruit	1 week at 70°F.				2 weeks at 70°F.				3 weeks at 70°F.			
			SER*	Pen.	Misc.	Total Decay	SER	Pen.	Misc.	Total Decay	SER	Pen.	Misc.	Total Decay
			Percent								Percent			
Check in water	11	550	6.9	0.4	0	7.3	53.8	2.7	0.4	56.9	70.2	3.1	0.5	73.8
5% 2-AMP in water	11	550	2.4	0	0.1	2.5	2.5	0.7	0.4	3.6	3.8	1.6	0.4	5.8
10% 2-AMP in water	11	550	1.3	0	0.1	1.4	2.0	0.4	0.3	2.7	2.5	1.3	0.4	4.2
In wax emulsion check	11	550	8.0	0.2	0.2	8.4	56.4	0.9	0.3	57.6	73.8	2.0	0.6	76.4
5% 2-AMP in wax emulsion	11	549	1.3	0.2	0.1	1.6	2.0	1.1	0.5	3.6	2.5	2.7	1.2	6.4
10% 2-AMP in wax emulsion	11	550	0.2	0	0.2	0.4	0.7	1.1	0.2	2.0	0.9	2.6	0.7	4.2

* SER—Stem-end rot (*Diplodia natalensis* or *Phomopsis citri*).
Pen.—Green mold (*Penicillium digitatum*).
Misc.—Miscellaneous rots other than SER or Pen.

detected in a few of the dipped fruits, and it was not in sufficient amount in any test to be of commercial significance; in fact, the injury may have been due to some factor other than the antiseptic.

2-AMP at 80°, 100°, 125°F.: Still another series of experiments was run to determine whether the fungicidal properties of 2-AMP could be increased by raising the temperature of the treating bath from 80° to 125° F. The time of exposure was 2 minutes in all cases. Two water check temperatures, 80° and 125°, were selected since they represented the extreme limits at which the antiseptic was applied. Table 4 gives the different categories of decay cumulatively.

Again the miscellaneous decay, mostly side rot, was of no consequence since the maximum amounted to only 2.2 percent after a 21-day holding period; likewise green mold, which developed to some extent in all treatments, was of minor importance since the maximum after 3-weeks' holding was only 2.5 percent.

In the 80°F. water checks there was slightly more stem-end rot at the first, second, and third inspections, respectively, than at the corresponding inspection of the 125° check.

In the case of the fruit treated with 5 percent solution of 2-AMP, a progressive but slight decrease in stem-end rot was observed at each of the weekly inspections as the temperature of the bath was raised from 80° to 100°, and then to 125°F., the last temperature being that at which the usual commercial color-added or dye treatment is applied.

Various other tests with 2-AMP: Supplementary tests were set up to determine whether: (1) aqueous solutions of 2-AMP would deteriorate rapidly on standing; (2) it would mix with solutions used for the color-added treatment; (3) it would be compatible with the wax emulsion in common use; (4) a spreading agent would increase its efficacy; and (5) wrapping tissue, treated with this material would check decay.

Re-use of 2-AMP: Results from three tests indicate that an old (used) water solution of 5 percent 2-AMP was about as effective after one and two weeks' standing as when freshly prepared. The non-treated lots had 64.5 percent total decay; those treated with fresh solution developed 5.6 percent; and those treated with a 2-weeks-old solution, 6.5 percent in three weeks.

2-AMP in dye: The antiseptic apparently was not impaired when mixed with the "color-added" dye solution, nor was there any apparent change in the coloring properties of the dye, as indicated in two tests in which no decay developed in treated lots while 42 percent decay was noted in the check lots.

2-AMP with wax emulsion: The antiseptic mixed well with seven lots of wax emulsion used on fruit and retained usual decay-repressing properties.

2-AMP with spreading agent: Evidence obtained from four tests indicated that decay-inhibiting properties of 2-AMP were not increased by the addition of 0.1 percent of Vatsol O. T. The average of four tests gave 58.5 percent total decay in three weeks in untreated check lots, 7.8 percent in the lots treated with 5 percent 2-AMP in emulsion, and 9.0 percent where the spreader was added.

2-AMP in wrapping tissue: Plain wrapping tissue impregnated with a 10 percent solution of 2-AMP in isopropyl alcohol was moderately effective in checking decay in three lots of dead-ripe Seedling oranges in March as is shown by the average of three tests. After three weeks' holding the fruit in untreated wraps had developed 44.8 percent stem-end rot and 27.6 percent green mold, while that in treated wraps showed 9.8 percent stem-end rot and 5.8 percent green mold. Decay from all causes amounted to 73.7 percent in the check lots and 18.6 in the treated fruit. It is interesting to note that considerable rind injury characterized by scattered brown, slightly sunken spots developed slowly in each of the lots wrapped in treated tissues. This blemish was not noticeable until after the first week.

TABLE 3—Decay Control in Gassed Oranges Treated with 5 Percent 2-Amino-Pyridine in Water for Varying Lengths of Time.

Treatment	No. Tests	No. Fruit	1 week at 70°F.				2 weeks at 70°F.				3 weeks at 70°F.			
			SER	Pen	Misc.	Total Decay	SER	Pen.	Misc.	Total Decay	SER	Pen.	Misc.	Total Decay
			Percent				Percent				Percent			
Water check 10 seconds	11	548	4.9	0	0	4.9	47.4	1.6	0.3	49.3	67.7	2.4	0.2	70.3
2-amino-pyridine 10 seconds	11	548	1.8	0	0.2	2.0	2.6	0.5	0.9	4.0	4.0	1.6	1.7	7.3
2-amino-pyridine 1 minute	11	549	1.6	0	0.6	2.2	1.8	0.9	0.8	3.5	4.6	1.5	1.0	7.1
2-amino-pyridine 3 minutes	11	550	1.8	0	0	1.8	2.9	0.4	0.7	4.0	4.7	1.6	1.7	8.0

* SER—Stem-end rot (*Diplodia natalensis* or *Phomopsis citri*).
Pen.—Green mold (*Penicillium digitatum*).
Misc.—Miscellaneous rots other than SER or Pen.

TABLE 4—Decay in Gassed Oranges Treated with 5 Percent 2-Amino-Pyridine Solution in Water (2 Minute Dip, no Subsequent Rinsing).

Temperature of treatment variable.

Treatment	No. Tests	No. Fruit	1 week at 70°F				2 weeks at 70°F				3 weeks at 70°F			
			SER*	Pen.	Misc.	Total Decay	SER	Pen.	Misc.	Total Decay	SER	Pen.	Misc.	Total Decay
			Percent				Percent				Percent			
Water check 80°F.	11	558	6.3	0.4	0.1	6.8	48.7	1.4	0.4	50.5	71.3	1.8	0.6	73.7
Water check 125° F.	11	526	3.4	0.2	0	3.6	39.0	1.0	0.1	40.1	65.0	1.5	0.2	66.7
2-amino-pyridine 80°F.	11	555	1.6	0	0.4	2.0	2.7	0.2	0.7	3.6	3.4	0.9	2.2	6.5
2-amino-pyridine 100°F.	11	557	1.1	0.2	0.3	1.6	1.8	0.9	0.9	3.6	2.7	2.5	1.6	6.8
2-amino-pyridine 125°F.	11	556	0.5	0.4	0	0.9	0.7	0.7	0.2	1.6	1.4	1.4	0.8	3.6

* SER—Stem-end rot (*Diplodia natalensis* or *Phom opsis citri*).
Pen.—Green mold (*Penicillium digitatum*).
Misc.—Miscellaneous rots other than SER or Pen.

Somewhat later, during the Valencia season, plain wrapping tissue was treated with 5 percent and 10 percent 2-AMP in wax emulsion. After drying, these wraps were used with three lots of fruit.

After 21 days 73.3 percent decay from all causes had developed in the check fruit. Of this 72.0 percent was stem-end rot and 1.3 percent green mold. In the same length of time 10.7 percent stem-end rot, 2.0 percent green mold, and 0.6 percent decay from all other causes appeared in the fruit wrapped with tissue dipped in wax emulsion containing 5 percent 2-AMP.

The control of decay in fruit covered with tissue impregnated with 10 percent 2-AMP in wax emulsion was no greater than with that in the wraps with the weaker dosage, to wit, there was 14 percent rot from all causes, of which 13.3 percent was stem-end rot and 0.7 percent green mold.

Inoculation experiments with 2-AMP and with sodium ortho-phenyl-phenate. Inoculation experiments were initiated in which freshly wounded oranges were inoculated with spores of the green mold fungus and later treated at intervals with a 5 and a 10 percent solution of 2-AMP in wax emulsion. In a similar series of experiments 1 1/4 percent sodium ortho-phenyl-phenate was added to the wax emulsion. The results of the tests, conducted under more extreme conditions than are likely to prevail in commercial practice, are given in Table 5.

Under these extreme conditions fruit treated with 10 percent 2-AMP in wax emulsion within a few hours after inoculation held up well for nine days. A progressive decrease in decay repression was noted with the increase of the time interval between inoculation and antiseptic treatment. When the concentration of 2-AMP was reduced to 5 percent its mold inhibiting properties were greatly weakened.

Under the same extreme conditions 1 1/4 percent sodium ortho-phenyl-phenate failed to give a lasting protection against green mold development, although it retarded spoilage more effectively than 5 percent 2-AMP. It is interesting to note that when the antiseptic was applied 8 hours after inoculation, the repression of decay was more effective than when the application was made at a greater or less interval after inoculation. In none of the separate tests was the decay development 9 days after inoculation greater in the lots receiving the antiseptic 8 hours after inoculation than in those treated 4 hours after. There seems to be no satisfactory explanation for this phenomenon, which occurred on 9 separate occasions.

Discussion

The need for some effective means of checking decay in Florida citrus fruit becomes obvious when it is seen that decay developed in approximately 50 percent of the untreated oranges in 2 weeks. While this rate is abnormal, it is sometimes encountered in commercial operations, especially when the fruit is ripe.

Inasmuch as 2-AMP is a derivative of pyridine, which in turn is found in considerable quantities in bone oil, there may be some basis for assuming that it may prove to be acceptable material for treating fruit for the prevention of decay.

For these tests no fruit drying facilities were available so all treated lots were placed in trays and set in the open air to dry. Sometimes the fruit dried rather quickly, but at other times, especially in cloudy or inclement weather, the drying time was several hours. Therefore, since injury is probably related to the length of time the fruit remained wet with the solution, it is probably unwise to place much emphasis on the rind injuries or to draw conclusions relative to the danger thereof in treatments given under commercial handling conditions.

The 2-AMP antiseptic mixes well with the general run of wax emulsions commonly applied to citrus fruits, as well as with

water. Used solutions retained their effectiveness after standing for at least 2 weeks. An effective application can be made by passing the fruit through a small tank containing 5 percent solution 2-AMP in wax emulsion or in water, or probably by flooding the solution on fruit for a few seconds in order to assure a thorough coverage. The material also is compatible with the dye used in the color-add treatment.

The evidence presented herein, although not closely paralleling that reported by E. F. Hopkins and K. W. Loucks in the June, 1947, issue of *Citrus Industry,* tends to substantiate their findings. These two investigations were conducted almost simultaneously but independently and with fruit from different sources and under different conditions.

Conspicuous absence of green mold rot in the Valencias was not unexpected, since the prevalence of this rot regularly diminishes in the spring and summer. There appears no basis for assuming that the Valencia orange is less susceptible to green mold than the winter-ripening varieties which fall a ready prey to it. The decline in the incidence of green mold in the spring seems to be associated with warmer weather and perhaps with less favorable humidity conditions.

A ten percent concentration of 2-AMP was effective in checking green mold in inoculated oranges, but neither 5 percent 2-AMP nor 1 1/2 percent sodium ortho-phenyl-phenate checked green mold rot in inoculated fruit satisfactorily under the conditions of the test; yet it is well known that the latter gives an excellent control of the rot under commercial conditions, which are far less harsh than those set up for the tests reported herein.

TABLE 5—Development of Green Mold in Inoculated Oranges Treated With Wax Emulsions Containing 2-Amino-Pyridine and With Sodium Ortho-Phenyl-Phenate.

Treatment	No. Tests	No. Fruit	Hours between inoculation and Treatment	Days at 70°F.		
				3	6	9
				Percent infection		
Check emulsion	3	78	8	83.3	100.0	100.0
10% 2-amino-pridine	3	79	4	0	1.3	2.5
do	3	78	8	1.3	2.6	6.4
do	3	79	16	3.8	8.9	10.1
do	3	79	24	15.2	34.2	40.5
Check emulsion	6	228	8	94.3	100.0	100.0
5% 2-amino-pyridine	6	228	4	7.0	24.6	37.3
do	6	228	8	7.5	18.4	27.6
do	6	229	16	27.9	52.4	56.8
do	6	227	24	52.9	70.9	75.3
Check emulsion	3	150	8	99.3	100.0	100.0
1 1/4% Dow A*	3	150	4	1.3	14.7	26.0
do	3	150	8	0	9.3	18.0
do	3	151	16	2.0	24.5	29.1
do	3	152	24	15.8	46.7	53.3

* Dow A—sodium ortho-phenyl-phenate.

Applying 2-AMP to wrapping tissues seems to be a promising method of application, especially since it does not bring about an attachment of the fungicide in a solid state to the fruit. Although 2-AMP has an odor, its presence on fruit could not be detected by smelling, nor did it affect the flavor or appearance of the juice of treated fruit.

SUMMARY

A 5 percent solution of 2-amino-pyridine (2-AMP) in water or in wax emulsion, applied to oranges after a 50 to 60-hour exposure to ethylene gas, gave very good to excellent control of decay in Florida oranges.

Plain wrapping tissue impregnated with 2-amino-pyridine was effective in checking decay in seedling and Valencia oranges.

Because of the relative absence of Penicillium rot during the period when the principal tests were made, the evidence of effectiveness against that fungus is not so striking as against the stem-end rot fungi.

Limited evidence based on inoculations indicates that weak concentrations of 2-AMP may not be quite so effective as sodium ortho-phenyl-phenate against green mold for a short period. 2-AMP did not affect the flavor or appearance of the juice of treated oranges.

The feasibility of commercial use of 2-AMP on citrus fruits, from the standpoint of possible toxic effects on consumers, has not as yet been determined.

PREVENTION OF ENTRANCE OF INSECT PESTS AND DISEASES FROM FOREIGN COUNTRIES

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It is unfortunate indeed that the speaker scheduled for this period, Mr. Arthur G. Watson, Assistant Collector of Customs, Tampa, Florida, is not able to be here and present a picture of foreign plant quarantine as viewed by one not primarily engaged in plant quarantine enforcement.

The activities of Customs and plant quarantine enforcement inspectors are closely related. The former are responsible for the regulation of entry of foreign commodities, largely from a revenue angle, while the latter are responsible for regulation of entry of plants and plant products to protect the agricultural interests of the United States from economic losses, sometimes of serious proportions, likely to follow entry of affected plants, fruits, etc., from foreign countries. As a matter of fact, rigid enforcement of Customs regulations would deny plant quarantine inspectors the right to board any newly arrived air-or water-craft, or even inspect plants until after Customs had completed entry of the craft and officially disposed of its cargo. It is apparent that a procedure of this nature would seriously interfere with the efficient application of plant quarantine regulations.

Mr. Watson, a grove owner himself, has been intensely interested in foreign plant quarantine enforcement since the inception of this branch of the Plant Board's activities in 1916 and has done everything within his authority to provide for the closest cooperation of Customs inspectors. This has resulted in a situation whereby the number of plant quarantine inspectors at Florida ports of entry totals not the number of Plant Board employees assigned any particular port, but the combined number of Plant