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Postharvest Benomyl and Thiabendazole Treatments, Alone and With Scald Inhibitors, to Control Blue and Gray Mold in Wounded Apples[1]

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Abstract. Benomyl and thiabendazole (TBZ), at concn of 1,000 ppm, were compatible with commercial scald inhibitors (2,700 ppm ethoxyquin or 2,000 ppm diphenylamine). No injury was observed on 'Delicious' or 'Stayman' apples given combined treatments and stored 5 months at 0°C plus 6 or 7 days at 21°C. Effectiveness of fungicide and scald inhibitor was not altered when combined.

Both benomyl and TBZ used as 10-15 sec dip treatments at 500 ppm controlled decay due to blue mold (*Penicillium expansum*) and gray mold (*Botrytis cinerea*) at puncture wounds in inoculated apples. They were less effective in controlling decay at bruises unless suspensions were heated in a range of 29°-45°C (84°-113°F) and used as a 2-min dip. Unheated benomyl was more effective than unheated TBZ in reducing blue mold at bruises. TBZ was less effective in controlling decay at punctures when treatment was delayed 24 hr after inoculation. TBZ added to water contaminated with blue mold spores, as in a dump tank, controlled decay at skin punctures but not at bruises during subsequent storage. Neither benomyl nor TBZ controlled Alternaria rot, which often developed at punctures when blue and gray mold rot were controlled.

Postharvest use of new systemic fungicides on apples offers excellent promise for reducing losses from blue mold (*Penicillium expansum* Lk. ex Thom) and gray mold (*Botrytis cinerea* Pers. ex Fr.), major causes of spoilage during storage and marketing in the USA. Now that apples are commonly dumped and handled in water and dunked or drenched with scald inhibitors, the chance of washing blue and gray mold inoculum into wounds is high. Blanpied and Purnasiri (1, 3) recently showed that the spore load in packing house water tanks is high and builds up as more and more apples are submerged. Recent examples of increased blue mold rot of apples following treatment in scald-inhibitor tanks have been published (6, 7).

Suspensions of 2-(4'-thiazolyl)benzimidazole (thiabendazole or TBZ) and methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl) are being widely tested on horticultural crops. They appear to be more effective against blue mold than other commercial fungicides. Now that an experimental registration for use of benomyl on pome fruits has been issued, with a temporary residue tolerance of 7 ppm, commercial trial and usage may be rapid. TBZ, cleared for use on citrus and bananas, still awaits approval by the Environmental Protection Agency for use on apples. Unheated TBZ and benomyl apparently are harmless to apple skin and leave no visible residue. Neither material affects respiratory activity of apples (6).

Benomyl and TBZ control blue mold rot and some other rots in pears (9, 13) and apples (2, 4-7, 10, 11, 14, 15). For most effective decay control, fungicides should supplement good refrigerated storage. Both fungicides controlled blue mold on punctured and inoculated apples, but were less effective on bruised apples, unless suspensions were heated (14, 15). Bruising, which damages lenticels and leaves them open for infection, is an important factor in the development of blue mold rot (16). Heated TBZ or benomyl (3 min 45°C) were effective at concn of 100, 250, or 500 ppm in controlling blue mold of inoculated bruised apples without apparent injury (14). Dipping fruit for 45 sec in TBZ or benomyl heated to 54.5°C has sometimes caused a scald-like injury (15) similar to that found on apples treated with warm water for scald control (8). Treatment in 45°C water alone will control Gloeosporium rot of apples but increases physiological disorders (12).

Some data are available on effectiveness of TBZ and benomyl when applied with a scald inhibitor. Good decay control with combined treatments was obtained on 'McIntosh' (6) and 'Stayman' (7) apples, but the latter cv. was injured by fungicide-diphenylamine combinations.

Objectives of the current studies during 2 seasons were to determine effectiveness of benomyl and TBZ in controlling

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Table 1. Blue mold decay in punctured 'Delicious' apples, inoculated and dipped in various fungicides and scald inhibitors, then stored 3 months at 0°C followed by 1 week at 21°C (1969 season).[z]

Postharvest treatment (10 sec)[y]	Decay at puncture (%)		
	3 mos 0°C	3 mos 0°C + 7 days 21°C	Treatment means[x]
Water check	97.2	99.0	98.1a
2,000 ppm DPA	93.3	97.6	95.4a
2,700 ppm SS	96.3	99.5	97.9a
2,400 ppm captan	62.5	79.8	71.2b
2,400 ppm captan + 2,000 ppm DPA	64.3	84.8	74.6b
2,400 ppm captan + 2,700 ppm SS	70.5	87.7	79.1b
1,000 ppm benomyl	3.5	6.5	5.0c
1,000 ppm benomyl + 2,000 ppm DPA	2.8	6.2	4.5c
1,000 ppm benomyl + 2,700 ppm SS	5.7	8.8	7.2c
1,000 ppm TBZ	2.7	4.7	3.7c
1,000 ppm TBZ + 2,000 ppm DPA	4.0	6.3	5.2c
1,000 ppm TBZ + 2,700 ppm SS	2.3	4.2	3.2c

[z]Data for each treatment based on 3 replicates of 100 apples with each replicate from a different orchard. One puncture wound per apple.

[y]Water and chemical dips were at 21°-23°C. DPA used was a 28% liquid concentrate.

[x]Treatment means followed by no letter in common are significantly different at the 1% level.

decay at punctures and bruises when: a) Combined with scald-inhibitor treatments, b) added to contaminated water as in a fruit dump tank, c) treatment is delayed after inoculation, d) treatment is given to freshly harvested fruit vs. fruit stored several months, e) suspensions are heated, and f) fruit is inoculated with gray mold.

Materials and Methods

Apple samples. Fancy or Extra Fancy size 113 'Delicious' apples were obtained from 3 or 4 nearby orchards with each orchard serving as a replicate. Fruit was randomized and a 100-fruit sample from each orchard was used for each treatment. Main tests were initiated in October of 1969 and 1970 with freshly harvested fruit and in January 1971 with fruit stored 4 months at 0°C. Initial apple firmness with the Magness-Taylor pressure tester averaged 14.5 lb. in the October tests and 11.5 lb. in the January tests. Fruit temp when treated with fungicides was 7°-10° in October and 2°-4°C in January. After treatment apples were packed in tray-pack boxes of 100 and stored at 0°C with 85% relative humidity in a randomized arrangement until examined after 2 to 5 months. Apples were transferred from 0°C to 21°C and re-examined after 6 or 7 days. 'Golden Delicious' and 'Stayman' apples were used in 1 experiment each.

Wounding of apples. Apples were punctured or bruised by pressing the side against the head of a finishing nail or the convex head of a thumbtack, respectively, imbedded in a board as previously described (15). When both types of wound were studied, a puncture was made on 1 side and a bruise on the opposite side.

Inoculation and treatment. In experiments with blue mold, fruit was inoculated immediately after wounding by dipping in a spore suspension made from 5-7 day old *Penicillium* cultures freshly isolated from stored apples and grown on potato-dextrose agar. Spore concn in the suspension, prepared with DuPont's Surfactant F (0.5 ml/l), was estimated with a haemocytometer to be 250,000 spores/ml in Oct. 1969 and Jan. 1971 tests and 440,000 spores/ml in Oct. 1970 tests. In an experiment with gray mold, the inoculum contained 50,000 *Botrytis* spores/ml. Sufficient inoculum was prepared to provide fresh spore-suspension for each 300-400 apples. The apples were then allowed to dry for 1 hr at room temp. Wounded and

Table 2. Blue mold decay in punctured 'Delicious' apples, inoculated and dipped in various fungicides and scald inhibitors, then stored 5 months at 0°C followed by 6 days at 21°C (1970 season).[z]

Postharvest treatment[y]	Decay at puncture (%)		
	5 mos 0°C	5 mos 0°C + 6 days 21°C	Treatment means[w]
Water check	100.0	100.0	100.0a
Water check, 2 min 45°C	100.0	100.0	100.0a
2,000 ppm DPA	99.8	99.8	99.8a
2,700 ppm SS	99.8	100.0	99.9a
500 ppm benomyl	7.5	10.5	9.0defg
1,000 ppm TBZ	4.0	6.8	5.4fg
500 ppm TBZ	7.0	12.3	9.6defg
500 ppm TBZ delayed 24 hr	40.3	42.0	41.1b
500 ppm TBZ, 2 min 45°C	3.0	4.8	3.9g
500 ppm TBZ, 2 min 45°C delayed 24 hr	18.3	20.8	19.5c
250 ppm TBZ	14.5	18.8	16.6cd
250 ppm TBZ, 2 min 45°C	1.3	2.8	2.0g
1,000 ppm TBZ + 2,000 ppm DPA[x]	11.0	15.0	13.0cdef
500 ppm TBZ + 2,000 ppm DPA	13.0	15.3	14.1cde
1,000 ppm TBZ + 2,700 ppm SS	6.0	9.0	7.5efg
500 ppm TBZ + 2,700 ppm SS	10.3	14.3	12.3cdef

[z]Data for each treatment based on 4 replicate boxes from 4 growers, 100 apples per box. Mean based on 800 apples with 1 puncture wound per apple.

[y]Water and chemical dips were 21°-23°C for 15 sec unless otherwise specified. Fruit temp when treated (Oct. 7-8, 1970) was 7°-10°C.

[x]Chemley's experimental formulation of TBZ and DPA (L-277).

[w]Treatment means followed by no letters in common are significantly different at the 1% level.

Table 3. Blue mold decay in bruised 'Golden Delicious' apples, inoculated and dipped in unheated fungicides (21°C), then stored 2 months at 0°C followed by 1 week at 21°C (1969 season).[z]

Postharvest treatment (10 sec)	Decay at bruises (%) 2 mos 0°C	2 mos 0°C + 7 days 21°C	Treatment means[y]
Water check	32.0	39.2	35.6a
500 ppm benomyl	20.0	22.2	21.1bc
1,000 ppm benomyl	12.2	23.5	17.8c
500 ppm TBZ	34.0	40.2	37.1a
1,000 ppm TBZ	25.5	35.0	30.2ab

[z]Data for each treatment based on 2 replicates of 100 apples each with 2 bruises on each fruit (400 bruises). Fruit stored in poly-lined cartons.

[y]Treatment means followed by no letters in common are significantly different at the 5% level.

inoculated fruit were then dipped, using wire baskets, in unheated (21°C) or heated (up to 45°C) tap water or chemical suspensions for 10 sec to 2 min, as shown in the tables, before packing and storing.

For tests with water contaminated with macerated blue mold-rotted apples, wounded fruit was not inoculated before treatment. Rather it was inoculated during submersion in the contaminated water, tested with and without TBZ or benomyl, simulating use of a fungicide in a dump tank. The blue mold spore count in the contaminated water was 47,000 spores/ml in an October test and 100,000 spores/ml in a January test.

Chemical suspensions were prepared in 20 liters of tap water on an active-ingredient basis using benomyl as a 50% wettable powder (WP), thiabendazole (TBZ) as a 60% WP, captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) as a 50% WP, ethoxyquin (Stop Scald or SS) (1,2,dihydro-6-ethoxy-2,2,4-trimethylquinoline) as a 70% emulsion, and diphenylamine (DPA) as an 83% WP and as a 28% liquid concentrate (LC).

Results and Discussion

Blue mold decay at punctures. Punctured and inoculated 'Delicious' apples dipped in water developed high amounts of blue mold decay (97-100%) during 3 or 5 months storage at 0°C (Tables 1 and 2). The lesions were large on removal from storage ranging from 2.5-5 cm in diam. During 6 or 7 additional days at 21°C, lesions grew and enveloped 1/3-1/2 of each fruit. Dipping similar apples for 10 or 15 sec in benomyl or TBZ before storage gave excellent protection from blue mold. Captan at 2,400 ppm reduced blue mold decay but did not give effective control. Both benomyl and TBZ were very effective at 500 or 1,000 ppm (Table 2). A TBZ dip at 1,000 ppm was significantly more effective in controlling decay on punctured apples than one of 250 ppm. However, 250 ppm TBZ used as a 2-min heated dip (45°C) was as good as any treatment with only 2% decay.

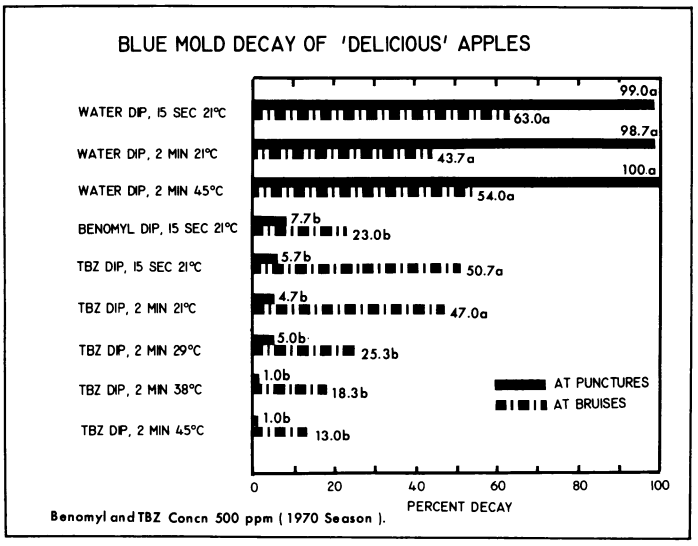

Fig. 1. Effect of unheated and heated water, benomyl, and TBZ postharvest treatments on decay of apples inoculated with *Penicillium expansum* spores at puncture and bruise wounds and stored for 2 months at 0°C plus 6 days at 21°C. Data based on 300 apples per treatment with 1 puncture and 1 bruise on each apple. Duncan 1% letters of significance are shown separately for punctures and bruises.

Delayed treatment with 500 ppm TBZ 24 hr after inoculation greatly reduced its ability to control decay of punctured apples.

Treating 'Delicious' with benomyl or TBZ in January after 4 months' storage, to simulate later marketing, also gave good control of blue mold during subsequent storage and holding. Fruit punctured, inoculated, and dipped in water in January developed 99% decay during 2 months at 0°C plus 6 days at 21°C (Fig. 1). Punctured fruit dipped 15 sec in 500 ppm benomyl or TBZ developed only 7.7 and 5.7% respectively. Use of various heated TBZ suspensions as 2-min dips did not significantly improve control over that obtained on punctured fruit with 15-sec unheated dips.

Fruit firmness after storage plus 6 days at 21°C was similar for fruit previously dipped in water, benomyl, or TBZ (data not shown).

Blue mold decay at bruises. Bruised and inoculated 'Delicious' and 'Golden Delicious' dipped 10 or 15 sec in unheated benomyl (500 ppm) developed less decay during storage than apples dipped in water (Fig. 1 and Table 3). Unheated TBZ, in contrast, failed to significantly reduce decay at bruises. Fewer blue mold lesions developed at bruise wounds

Table 4. Blue mold decay in punctured and bruised 'Delicious' apples dipped in contaminated water with and without thiabendazole, then stored at 0°C followed by 6 days at 21°C (1970 season).[z]

Postharvest treatment (15 sec)	Decay (%), test 1 5 mos 0°C	5 mos 0°C + 6 days 21°C	Decay (%), test 2 2 mos 0°C	2 mos 0°C + 6 days 21°C	Mean decay (%)[y]
Decay at puncture:					
Contaminated water	99.3	99.8	84.3	100.0	96.4a
Contaminated water with 500 ppm TBZ	2.5	5.3	0.0	3.3	2.9b
Decay at bruise:					
Contaminated water	—	—	32.7	37.0	34.8a
Contaminated water with 500 ppm TBZ	—	—	27.7	36.0	31.8a

[z]Data based on 4 replicate boxes from 4 growers in test 1 (treated Oct. 1970), and 3 replicate boxes from 3 growers in test 2 (treated Jan. 1971), 100 apples per box. Apples punctured on 1 side in both tests and bruised on the opposite side in test 2 before the 15-sec submersion. Contaminated water contained macerated blue-mold rotted apple tissue giving inoculum of 47,000 spores/ml (test 1) and 100,000 spores/ml (test 2).

[y]Treatment means within blocks followed by no letter in common are significantly different at the 1% level.

Table 5. Gray mold decay in 'Delicious' apples removed from storage, wounded, inoculated and dipped in water or fungicides, then stored 2 months at 0°C followed by 6 days at 21°C (Jan. 28 to April 6, 1971).[z]

Postharvest treatment (15 sec)	Gray mold decay (%) 2 mos 0°C	2 mos 0°C + 6 days 21°C	Treatment mean[y]
Decay at puncture:			
Water check	81.3	96.3	88.8a
500 ppm TBZ	10.7	19.7	15.2b
500 ppm benomyl	2.3	13.0	7.7c
Decay at bruise:			
Water check	7.7	11.0	9.3a
500 ppm TBZ	6.7	7.0	6.8ab
500 ppm benomyl	2.0	2.3	2.2b

[z]Data for each treatment based on 300 fruit (3 replicate boxes from 3 growers with 100 apples per box). Apples were punctured on 1 side and bruised on the opposite side and inoculated in a *Botrytis* spore suspension (50,000 spores/ml). Fruit temp when treated 7° to 10°C.

[y]Treatment means followed by no letters in common are significantly different at the 5% level.

than at skin punctures but bruises were a major source of entry for spores and subsequent spoilage.

Heated suspensions of benomyl and TBZ for 45 sec at 54.5°C or 3 min at 45°C previously were reported to improve decay control at bruises (14, 15). In the present study, dipping bruised apples for 2 min in water at 45°C did not reduce blue mold (Fig. 1). Heated TBZ (2 min 45°C), however, was significantly better than unheated TBZ in controlling blue mold at bruises. Use of TBZ heated only to 29°C was also more effective against decay at bruises than use of room temp (21°C) suspensions. The authors previously reported that more fungicide is deposited on fruit dipped at 45°C than at 21°C (14).

No heated suspensions of fungicides should be used after waxing fruit since the heat smears the wax and damages appearance. Use of a heated fungicide, if considered practical, would increase fruit temp and the added heat load would have to be removed. Thermocouple measurements of warm apples dipped 1 to 3 min in 45°C water showed that temp at the core increased from 20°C initially to 23°C, and temp just beneath the skin increased from 20°C to 38°-41°C.

Fungicide scald-inhibitor combinations. Suspensions of fungicides and scald inhibitors were prepared without noticeable chemical incompatibility. The pH of the suspensions alone and combined, ranged from 7.2 to 8.0. The fungicidal activities of captan, benomyl, and TBZ were not changed in the presence of either diphenylamine (DPA) or ethoxyquin (SS). The scald inhibitors alone had no effect on extent of decay of punctured and inoculated 'Delicious' apples. In contrast, either benomyl or TBZ at 1,000 ppm when combined with 2,000 ppm DPA or 2,700 ppm SS effectively controlled blue mold of punctured apples without apparent injury (Tables 1 and 2). Both 28% LC and 83% WP forms of DPA were used satisfactorily with the fungicides. Fungicidal activity of TBZ at either 500 or 1,000 ppm when combined with DPA or SS was good on 'Delicious' apples stored up to 5 months (Table 2). Little scald developed on these fruit, either treated or non-treated, during storage or after removal.

In tests with 'Stayman' apples, inoculated but without artificial punctures, combined treatments of 1,000 ppm benomyl or TBZ with DPA or SS reduced blue mold and controlled scald. 'Stayman' apples dipped in tap water, benomyl, or TBZ averaged 37% scalded fruit after 5 months at 0°C plus 7 days at 21°C. Fruit dipped 10 sec in a combined fungicide scald-inhibitor bath before storage developed 0 to 1% scald (data not shown). Again, there was no injury from use of combined scald-inhibitor fungicide on this cv. and no interference with fungicide or scald-inhibitor activity when combined. Addition of benomyl or TBZ to a scald-inhibitor immersion or drench operation before storage appears to be highly beneficial.

Blue mold from contaminated water. Some inoculation of apples with spores in dump tanks or during handling in water is inevitable even with efforts at improved sanitation (1, 3, 6). Sometimes chlorine or sodium-o-phenyl phenate is used to reduce postharvest decay (10), but better fungicides are needed. To test the effects of adding a fungicide to water contaminated with blue mold spores, 'Delicious' were immersed before and after the addition of 500 ppm TBZ. TBZ was strikingly effective in reducing blue mold decay at puncture wounds during storage, from 96% with no fungicide in the contaminated water to 3% with the fungicide (Table 4). These tests were with both freshly harvested fruit and with fruit from storage in January.

Addition of TBZ at 500 ppm to contaminated water did not reduce blue mold decay of bruised apples, following immersion and subsequent storage.

Gray mold decay. Decay from gray mold contamination is another important cause of apple losses in storage. To test the value of TBZ and benomyl in reducing this rot, 'Delicious' taken from storage were punctured on 1 side and bruised on the other and immediately inoculated in a *Botrytis* spore suspension, then dipped 15 sec in water or the fungicides (Table 5). Both fungicides were effective in controlling gray mold at punctures during 2 months at 0°C plus 6 days at 21°C. This substantiates a previous report on TBZ with 'McIntosh' and 'Cortland' (4). However, benomyl was slightly more effective than TBZ.

Relatively little gray mold developed at inoculated bruises on check fruit and TBZ was ineffective in reducing decay. Use of 500 ppm benomyl as a post-harvest treatment significantly reduced gray mold decay at bruises during storage.

Alternaria decay. Other studies have shown that neither TBZ nor benomyl controlled rot due to *Alternaria* sp. on pears or apples (4, 13). No artificial inoculation with this pathogen was done in this study. However, Alternaria decay developed in 10 to 15% of the puncture wounds in 'Delicious' apples but was largely absent in bruises. This rot was not apparent on removal from storage, but developed markedly during 6 days at 21°C on apples where blue and gray mold were controlled by benomyl or TBZ. None of the fungicides tested controlled Alternaria rot. Thus, Alternaria decay may occasionally be a problem even if blue and gray mold are controlled with benomyl or TBZ. Fortunately, *Alternaria* is a much less serious cause of loss than *Penicillium* and *Botrytis*.

Benomyl and thiabendazole appear very effective for postharvest control of blue mold and gray mold decay of apples. This is particularly true at puncture injuries but less so at bruises, unless fungicides are heated. Benomyl now has an experimental clearance for commercial trial, but thiabendazole must await clearance by the U. S. Environmental Protection Agency for use on apples.

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Propagation of Asparagus Through Shoot Apex Culture. I. Nutrient Medium for Formation of Plantlets[1]

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Abstract. A nutrient medium which enabled rapid formation of new spears and roots in shoot apices excised from buds as well as lateral branches of *Asparagus officinalis* L. spears was developed. This medium was composed of the following, in mg/1: Murashige and Skoog's inorganic salts; NAA, 0.3; kinetin, 0.1; thiamin·HCl, 1.0; pyridoxin·HCl, 5.0; *myo*-inositol, 100; adenine sulfate·dihydrate, 40; sucrose, 25,000; Difco Bacto malt extract, 500; $NaH_2PO_4 \cdot H_2O$, 170; and Difco Bacto agar, 6000. The shoot apices were cultured under 1000 lux Gro Lux or Plant Gro light and at constant 27°C. The explants were 0.15 mm in height and composed of the apical meristem plus a few visible subjacent primordial leaves. Within 6 weeks an avg of 80-90% of the cultures developed into miniature plants with several spears and roots. These plants, however, could not be transferred to soil with much success. The transfer necessitated further culture under another set of conditions, details of which are currently under investigation. The nutrient medium was inapplicable to shoot apex cultures of *A. densiflora* (Kunth) Jessop cv. Meyers, *A. densiflora* (Kunth) Jessop cv. Sprengeri, and *A. sarmentosus* (Hort.).

Plant cell and organ cultures are becoming increasingly popular and important in horticultural research and in plant propagation. Major effort in this laboratory has been directed towards their application in propagation, pathogen exclusion, and hybridization. We reported previously on progress in asexual multiplication of asparagus through callus cultures derived from spear slices (Takatori, Murashige and Stillman, 1968). Other investigators described attempts to propagate asparagus from stem tips (Andreasson and Ellison, 1967; Gorter, 1965) and cell suspension cultures (Wilmar and Hellendoorn, 1968). Unfortunately, success in these instances has been inconsistent. Moreover, a high frequency of plants derived from callus or cell culture deviated genetically from the original plants (Malnassy and Ellison, 1970). Research with diverse plant species has established that endopolyploidy and other genetic aberrations occur quite regularly in callus and related cell cultures (Murashige and Nakano, 1965, 1967; Partanen, 1963). To overcome the inconsistency and to avoid genetically aberrant plants our recent efforts were turned toward the use of tissue cultures started from minute shoot apices.

Success thus far has been more consistent through shoot apex cultures and, in contrast to callus cultures, fewer polyploid plants are expected, since the constituent cells of the shoot apex are less differentiated and more uniformly diploid than those of the mature plant parts (D'Amato, 1952; Partanen, 1955).

Materials and Methods

Field grown plants of the edible asparagus, *Asparagus officinalis* L., were employed as source of explants. The cv. 66 was utilized in most experiments. Formal tests disclosed that shoot apex explants from either the buds or the tips of young lateral branches of spears were satisfactory. All buds or laterals of a spear were usable.

To obtain explants from buds the outermost scale leaf was removed, and the exposed bud, together with a 2-mm thick by 8-mm long section of the adjoining stem tissue, was detached from the spear. This material was surfaced-sterilized and trimmed further to obtain the shoot apex. With outgrowing laterals the preliminary excision step involved removal of 1-cm sections of the shoot tip and their surface disinfection.

The detached sections were wrapped in small sheets of cheese cloth, in groups of 10 sections per sheet, transferred to 25 x 150 mm culture tubes and immersed in dilute laundry bleach for 10 min. Commercial bleach diluted to contain 0.5% sodium hypochlorite was utilized. Tween 20 emusifier was added at the rate of 0.1% to enhance spread of disinfectant. The tubes containing tissue sections and disinfectant were capped with polypropylene closures during the course of sterilization. The tissue was rinsed 3 times with autoclaved distilled water to remove the disinfectant.

Shoot apices were isolated for culture by adhering to aseptic procedures, and working under 12 X magnification. The explant contained the apical meristematic dome and a few leaf primordia. Each isolate measured approx 0.15 mm in height. Experience in this laboratory has shown that, whereas larger explants resulted in a higher frequency of survival, smaller explants possessed a greater tendency to root and form complete plants. Large explants resulted in only multiple spears and callus.

The basal medium, or the initial reference nutrient medium,

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