

Plant Growth Regulating Chemicals

Volume II

Editor

Louis G. Nickell, Ph. D.

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Vice President

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Velsicol Chemical Corporation

Chicago, Illinois



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PREFACE

The need to increase the world food supply substantially by the end of this century poses one of the greatest challenges yet faced by man. Many agricultural scientists believe that this challenge can be met, and it is expected that plant growth regulators will play an increasingly important role in meeting this challenge.

Plant growth regulating chemicals are used to modify crops by changing the rate or pattern or both of their response(s) to the internal and external factors which govern all stages at crop development from germination through vegetative growth, reproductive development, maturity, and senescence or aging, as well as post-harvest preservation.

The purpose of this two-volume work is to make available both to the investigator and to the user, on a crop by crop basis, the latest information on the use of chemicals to regulate plant growth and development. Emphasis is given to the major crops and to those with which the most success has been achieved. Since the degree of practical success with each crop varies, primary attention is given to chemicals registered for specific use(s) with the particular crop discussed. Also included is information concerning chemicals not yet registered, but for which practical results are available. In some cases information concerning active compounds in the exploratory stages is included. Where known and pertinent, information concerning mode of action is included.

The obvious classifications to use in presenting data on effectiveness of plant growth regulating chemicals are (1) by crop, (2) by chemical class, and (3) by plant function or process. Essentially all major summary or survey publications to date have been based on the plant function or process approach. This is primarily an academic approach and is not nearly as useful for practical purposes as a presentation by crops, as is done in this publication.

THE EDITOR

Louis G. Nickell, Ph.D., Vice President of Research and Development, Velsicol Chemical Corporation, Chicago, Illinois, was born July 10, 1921, in Little Rock, Arkansas. He received his B.S. degree in botany from Yale University in 1942. After serving 4 years in the U.S. Marine Corps as a regular commissioned officer, he returned to Yale University, receiving his M.S. in microbiology in 1947 and his Ph.D. in plant physiology in 1949. He is married to Natalie Wills Nickell and has three children and four grandchildren. His first professional experience was as Research Associate at the Brooklyn Botanic Garden from 1949 to 1951 where he was engaged in research on plant tissue culture and plant growth substances. He joined industry in 1951, going to Pfizer, Inc. in Brooklyn as its Plant Physiologist and Assistant Mycologist. There he specialized in antibiotics and their effects in agriculture as well as plant tissue and cell culture. In 1953 he became Head of Pfizer's Phytochemistry Laboratory and received the first patent issued for the use of plant cell cultures for the production of secondary products. In 1961 he moved to Hawaii to become Head of the Plant Physiology and Biochemistry Department of the Hawaiian Sugar Planters' Association, becoming its director of research in 1965. His first commercial success with plant growth regulating chemicals was the registration of diquat for the prevention of flowering in sugarcane in the early 1960's. This was followed successively by the registration of gibberellic acid for increasing the sugar yields in cane and later by the development of the first commercial product for the ripening of sugarcane, glyphosine. In 1975, he joined the Research Division of W. R. Grace & Company as Vice President of its Research Division in charge of agricultural, biological, and medical research, development, and commercialization. In 1978, he joined Velsicol Chemical Corporation as Vice President of Research and Development — his present position. His publications (over 300) and patents (over 30) have been primarily in the area of plant cell and tissue culture and the regulation of plant growth through the use of chemicals. He is the author of a book published in early 1982 entitled *Plant Growth Regulators — Agricultural Uses*.

He has served as President of the Hawaii Academy of Science, as Chairman of the Hawaiian Section of the American Chemical Society, as Vice Chairman and Chairman of the Plant Growth Regulator Society of America, as Council Member of the Society for Economic Botany, and has been the Treasurer of the American Society of Plant Physiologists since 1976. He has served as Chairman of the Governor's Advisory Committee on Science and Technology in Hawaii, as a member of the National Academy of Sciences — National Research Council Committee on Agricultural Production Efficiency, and is Chairman of the "The Forward Edge" Session of CHEMRAWN II, the International Conference on Chemistry and World Food Supplies: The New Frontiers, and a member of the Editorial Board of the Journal of Plant Growth Regulation.

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Chapter 1

TROPICAL FRUIT AND BEVERAGE CROPS

D. P. Bartholomew and R. A. Criley

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I. INTRODUCTION

A wide variety of fruit and beverage crops are grown in the tropics but few of them are intensively cultivated using modern crop production practices. The commercial use of growth regulators on tropical fruit and beverage crops appears to be restricted to those crops that have been grown on sufficient scale to justify the research support required to evaluate crop responses to them.

The crops selected for this review are *Ananas comosus* L. Merr. (pineapple), *Carica papaya* L. (papaya, pawpaw), *Mangifera indica* L. (mango), *Musa* spp. (banana, plantain), *Psidium guajava* L. (guava), and the beverage crops *Coffea arabica* L., *C. canephora* L. (coffee), and *Theobroma cacao* L. (cacao). The above crops are either important in international commerce and, thus, are grown on large plantations, often controlled by multinational corporations (e.g., banana, cacao, coffee, pineapple), or are grown more or less intensively on a somewhat smaller scale to supply local and regional markets. World production in 1980 for the crops in 1000 metric tons are the following: pineapple — 7636, papaya — 1917, mango — 14,342, dessert bananas — 39,254, plantains — 21,265, guava — no data reported, coffee — 4821, and cacao — 1557.⁴ It is likely that the above figures do not include significant quantities of the fruit crops that are grown by home owners and on small farms for direct consumption or for local markets.

Growth regulators are used on the above crops to enhance or speed propagation, induce flowering, increase fruit size, promote ripening on the plant, and delay ripening after harvest. Much work has been directed towards induction of flowering for year-round production and on ripening to concentrate harvesting because fruit bearing tends to be seasonal, or biennial as in some mango cultivars, even though the environment may be suitable for production throughout the year. Commercial use of growth regulators has reduced costs and extended supplies of commodities with significant benefits to both the producers and consumer.

II. *Ananas comosus* (L.) Merr. (PINEAPPLE)

A. Propagation

Pineapple (*Ananas comosus* [L.] Merr.) is the only member of the family Bromeliaceae that is cultivated as a food crop. Pineapple is grown from about 30° south latitude through the equator to 30° north latitude. The major cultivar of commerce is the Smooth Cayenne and there are several clonal selections of that cultivar being grown in Hawaii and in other parts of the world. Several other cultivars of pineapple are grown,^{22,112,113,122} but none approach the prominence of Smooth Cayenne.

Pineapple is propagated exclusively by asexual means using the tops of fruits (crown), offshoots borne at the base of the fruit which morphologically are crowns of vestigial fruits⁷⁸ and are referred to as slips, and shoots which arise from buds that develop in the leaf axils (suckers), presumably as a result of the loss of apical dominance that occurs coincidentally with inflorescence initiation. Pineapple is not propagated by seed because cultivars must be highly self-incompatible to be commercially successful,⁸⁹ and cross pollination results in progeny which shows variability typical of heterozygous parents.

Because vegetative propagation dependent on the production of crowns, slips, or suckers is slow, various stem-sectioning techniques have been devised to increase the number of propagules.^{22,35,46,88,128,132,147} Pineapple also has been propagated by meristem budding techniques^{39,90,91,93,104,134,140,160} and plantlets have been regenerated from callus.^{92,115,146}

There is no indication that important clones have been mass propagated by tissue culture. In fact, a pineapple company in Hawaii abandoned attempts to propagate clones of pineapple by tissue culture techniques because variability was observed among the progeny. Others also have observed variants among pineapple plants propagated from tissue culture.^{92,146} Plantlets regenerated from syncarp tissue exhibited greater variability than did plantlets regenerated from meristem tissue of slip, crown, and axillary buds.¹⁴⁶ No studies were reported where tissue-cultured plantlets were grown to maturity to assess the effects of variability on productivity and fruit quality. Until such data become available, the mass propagation of pineapple clones by tissue culture techniques should be approached with caution. Tissue culture techniques may be most useful for the propagation of potentially valuable new clones produced in a breeding program so long as roguing is practiced to remove offtypes.

Rapid propagation of field-grown pineapple can be accomplished by spraying plants with the morphactin Multi-prop® (Celamerck GmbH & Co.) or Maintain CF125® (U.S. Borax Co.).¹²⁴ Multi-prop®, a mixture of methyl esters of 2-chloro-9-hydroxyfluorene-(9)-carboxylic acid (chlorflurenol), 9-hydroxyfluorene-(9)-carboxylic acid (flurenol), and 2,7-dichloro-9-hydroxyfluorene-(9)-carboxylic acid (dichlor-flurenol), induces the formation of plantlets, which are normal both in appearance and in growth rate, on the peduncle or on the developing inflorescence of pineapple.^{28,51,80,81,150} The morphactin mixture is applied as an aqueous spray over the plants after inflorescence development has been initiated (forced) with ethephon (2-chloroethylphosphonic acid), alpha-naphthaleneacetic acid (NAA), or its sodium salt (SNAA). The timing of application is determined by the effect of the prevailing environment on normal inflorescence development^{51,81} and by the number and final size of the plantlets desired. Plantlet number is also influenced by plant size with larger plants producing greater plantlet numbers than small plants.¹⁵⁰ Morphactin-induced plantlet number and mass were greater when plants were forced with ethephon or SNAA than with ethylene or beta-hydroxyethylhydrazine (BOH).⁵¹

In Australia, the application of Multi-prop® 1 to 4 weeks after forcing resulted in 10 to 30 plantlets per plant weighing an average of 200 to 50 g each when harvested 43 weeks after forcing.⁵¹ Application of Multi-prop® 1 week after forcing resulted in the production of more plantlets on the fruit peduncle, while later application increased those borne on the

fruit and reduced those borne on the peduncle.^{51,150} Sucker numbers were reduced by the application of Multi-prop® 7 days after forcing, but not to unacceptable levels. Where large plantlet numbers were produced, sucker growth was inhibited until the plantlets were harvested. The current recommendation for use in Australia is to force plants with ethephon followed by the application of 2500 to 3000 ℓ ha⁻¹ of a solution containing 22 ppm active ingredient (a.i.) of Multi-prop® 10 to 14 days after forcing in early May (forced for winter harvest).^{51,149} In South Africa,⁸¹ two applications of Multi-prop® have been made at 7- or 12-day intervals. Both 1.6 and 2.0 ℓ of the chemical (11.2% a.i.) were applied per hectare initially followed by 3.2 or 4.0 ℓ /ha. No data were given on the spray volume used. Timing of application after forcing was important with application being made sooner after forcing in summer than in winter. Results indicated that up to one million plantlets could be produced from 1 ha containing 43,000 ratooned pineapple plants.⁸¹ Chlorflurenol is used commercially in Hawaii and in Brazil but no data are available on specific practices.

Chlorflurenol is labeled as a poison but it has low toxicity to wildlife, fish, and mammals.⁵

B. Vegetative Growth

There is no commercial practice utilizing growth regulators to promote vegetative growth of pineapple nor were any published reports found suggesting that there is interest in, or potential for, developing such practices.

C. Induction of Flowering (Forcing)

1. History of Growth Regulator-Induced Flowering

The artificial induction of flowering in pineapple dates to about 1874 when it was accidentally discovered that wood smoke uniformly forced pineapple plants to flower.²² This discovery was exploited commercially in glasshouse culture of pineapple in the Azores²² and in field culture in Puerto Rico.^{42,119} Rodriguez¹¹⁹ showed that a component of smoke, ethylene, was as effective as smoke as a flower inductant. Acetylene gas, an acetylene-saturated solution of water, and calcium carbide, which releases acetylene on contact with water, were shown to force pineapple in the 1930s.^{82,83, 87} In 1941, Johnson showed that an ethylene-saturated water-oil emulsion containing colloidal earth could also force pineapple.⁷⁹

The events leading to the discovery that growth regulators with auxin activity could force pineapple apparently are not documented. Several years of research must have prefaced the first published reports in 1942 because data from Hawaii²⁰ were compared with data for IAA and NAA from Florida in the same year.²⁵ A few years later it was shown that 2,4-D also could induce flowering of pineapple and that Cabezona pineapple could be forced every month of the year.^{142,143}

Beta-hydroxyethylhydrazine (BOH) which is thought to be an ethylene releasing agent^{133,138} was reported to induce flowering in Smooth Cayenne in 1955.⁶⁴ More recently, ethephon (2-chloroethylphosphonic acid) and CGA-15281 (beta-chloroethyl-methyl-bis-benzyloxy-silane), which also degrade to release ethylene^{19,26} and stimulate the plant to produce ethylene,¹⁴⁸ have been shown to force pineapple.^{15,24,109}

Many other compounds have induced flowering in pineapple,^{41,55,65-68} but little or no additional work has been done with many of them and none is of commercial importance. The literature on those compounds studied in some detail is summarized in Table 1. Only four compounds — acetylene (and calcium carbide), ethephon, ethylene, and NAA (or SNAA) — have been used in commercial practice to force pineapple.

2. Absorption and Mechanism of Action

Green leaf tissue is required for flower initiation with gaseous ethylene.¹⁴¹ Flowering did not occur if only the basal portions of older leaves and the etiolated young leaves in the center of the plant were retained. Plants defoliated to one large green leaf before treatment or defoliated completely 2 days after treatment were induced to flower.¹⁴¹

Table 1
SUMMARY OF STUDIES ON THE USE OF GROWTH
REGULATORS AS FLOWER INDUCTANTS OF PINEAPPLE

Growth regulator	State, country	Cultivar	Ref.
Acetylene and calcium carbide	Australia	Smooth Cayenne	48, 87
	Brazil	Pernambuco	43, 94
		Perola	44
	Colombia	Smooth Cayenne	121
	Cuba	Red Spanish	53—55
		Smooth Cayenne	53—57
	Florida	Abachi	26
	Ghana	Sugarloaf	98—100, 102
		Smooth Cayenne	1, 101
	Guinea	Smooth Cayenne	107, 113
	Hawaii	Smooth Cayenne	83
	India	Kew, Giant Kew (Smooth Cayenne)	33, 70, 96
	Kenya		41
	Malaysia	Sarawak (Smooth Cayenne)	156
		Singapore Spanish	151, 153, 155
	Mexico	Smooth Cayenne	2
	Puerto Rico	Smooth Cayenne	135
	Sri Lanka (Ceylon)	Kew (Smooth Cayenne)	126
	South Africa	Smooth Cayenne	30
	Taiwan	Smooth Cayenne	75, 157, 158
Alpha-naphthalene-acetic acid (ANA, NAA, SNA)	Australia	Smooth Cayenne	48, 69, 97
	Bangladesh	Giant Kew (Smooth Cayenne)	3, 129
		Honey Queen	130
	Brazil	Pernambuco	43
	Florida	Abachi	25
	Ghana	Sugarloaf	98, 99
	Guinea	Smooth Cayenne	107, 111, 113
	Hawaii	Smooth Cayenne	20
	India	Kew, Giant Kew (Smooth Cayenne)	31, 32, 70, 114
	Kenya		41
	Malaysia	Singapore Spanish	155
	Mexico	Smooth Cayenne	2
	Puerto Rico	Cabezona	142—146
		Red Spanish	146
	South Africa	Smooth Cayenne	30
	Sri Lanka (Ceylon)	Kew (Smooth Cayenne)	126, 127
		Mauritius	127
	Taiwan	Smooth Cayenne	75, 158
Beta-hydroxy- ethylhydrazine	Australia	Smooth Cayenne	48
	Ghana	Sugarloaf	98—100
	Malaysia	Mauritius	19
	Mexico	Smooth Cayenne	2
	Puerto Rico	Smooth Cayenne	133
	Taiwan	Smooth Cayenne	158

Table 1 (continued)
SUMMARY OF STUDIES ON THE USE OF GROWTH
REGULATORS AS FLOWER INDUCTANTS OF PINEAPPLE

Growth regulator	State, country	Cultivar	Ref.
2,4-Dichlorophenoxy- acetic acid	India	Giant Kew (Smooth Cayenne)	76
	Puerto Rico	Cabezona	142, 143
		Red Spanish	142, 143
Ethephon	Peru		58
	Australia	Smooth Cayenne	48, 52
	Colombia	Smooth Cayenne	121
	Cuba	Red Spanish	53, 54
		Smooth Cayenne	53—57
	Ghana	Sugarloaf	98—100
	Guinea	Smooth Cayenne	109, 113
	India	Kew, Giant Kew (Smooth Cayenne)	34, 36, 70, 114
	Malaysia	Singapore Spanish	154
	Philippines	Smooth Cayenne	13, 14
	South Africa	Smooth Cayenne	30
	West Africa	Smooth Cayenne	71, 72, 109, 138
Ethylene	Australia	Smooth Cayenne	48
	Brazil		47
	Florida	Abachi	141
		Red Spanish	141
	West Africa	Smooth Cayenne	27, 37, 109
Indoleacetic acid	Florida	Abachi	25
	India	Smooth Cayenne	32
Naphthaleneacetamide	Hawaii	Smooth Cayenne	20
	Florida	Abachi	25
	India	Giant Kew (Smooth Cayenne)	114

It is not known whether acetylene and ethylene are absorbed through the leaf trichomes¹²⁰ and the cuticle, through the stomates, or all three. Evidence that forcing with unsaturated hydrocarbons is much more effective at night^{1,2,30,75,158} and in the absence of wind⁷⁵ indicates that the stomata are an important avenue for entry into the leaf. The effectiveness of night application is explained, at least in part, by the fact that pineapple is an obligate Crassulacean acid metabolism plant so the stomata are closed during the day, open in late afternoon, and remain open throughout the night.⁹

NAA and ethephon are rapidly absorbed and absorption is presumed to be through the trichomes, or cuticle, or both. Within 5 min after labeled NAA having an activity of 60,000 cpm was applied to a leaf, 200 cpm of activity was detected in the stem apex.⁸⁶ Rain following the application of NAA does not alter the induction of flowering.¹¹³ Rapid absorption of ethephon is demonstrated by the fact that flushing the plant with 6 ℓ of water from 1 min to 2 hr after application has no effect on forcing.⁵⁰

Flower induction with NAA and ethephon is a response to a specific quantity of growth regulator rather than to a volume or concentration.^{71,143} Forcing has been achieved by the application of 5.0 mg of ethephon in a 20- $\mu\ell$ volume into the plant heart or to a 1-cm² spot on a single leaf.¹⁰

The mechanism by which the various forcing agents initiate flowering in pineapple is not known. Basic research on the physiology of flower induction has not extended knowledge beyond the point where it is generally believed that induction occurs in response to ethylene.

Although Gowing hypothesized that NAA induced flowering in pineapple by acting as an antagonist of native IAA,⁶² more recent data suggest that flowering occurs as a result of ethylene production by the plant which is stimulated by NAA.¹⁷ NAA induced evolution of ethylene from pineapple attained a peak about 1 week after treatment¹⁷ and fruit from pineapple forced with NAA are harvested a week or more later than fruit from plants forced with acetylene, ethylene, or ethephon.^{30,98} The approximately 1-week delay in harvesting of fruit from plants forced with NAA may be due to a delay in the NAA forcing response. However, it was recently shown that bract and flower primordia development began at the same time in the Masmerah and Smooth Cayenne cultivars regardless of whether they were forced with ethephon or NAA.^{95,156} Significant amounts of ethylene are produced within 16 hr by pea (*Pisum sativum*)¹⁸ and mung bean (*Vigna radiata*)¹⁵⁹ tissue incubated in media containing IAA, so it is likely that sufficient ethylene is present at the apex of pineapple within a few hours after treatment with NAA to induce flowering. Therefore, it seems likely that the delayed maturation of pineapple fruits on plants forced with NAA is a side effect of auxin forcing.

Recently, silver ion, an inhibitor of ethylene action,¹² was found to inhibit flower induction of pineapple with ethephon,¹²³ further suggesting that ethylene is in some way involved in the induction process.

3. Plant Susceptibility to Forcing

In order to force pineapple into flower, a minimum plant size is required.^{14,23,33} Das et al.³³ found that a minimum of 12 leaves about 30 cm in length was required before fruit formation could be induced. In another study,²³ 2-month-old plants (514 g fresh weight, 62.9 g dry weight) could not be forced with ethephon regardless of the night temperature at which they had been conditioned. Four-month-old plants (878 g fresh weight, 114.6 g dry weight) were readily forced if the night temperature was controlled at 20°C.²³ In commercial practice the minimum plant size required for forcing is attained well before the plant is large enough to produce a fruit of marketable size and plant and fruit mass are highly correlated.^{33,36,45,83,87,97,110,137,143} The pineapple plant produces a terminal inflorescence so the initiation of new leaves ceases at the time that flower parts begin to be laid down. Because of this fact, comparisons of fruit characteristics of forced plants with plants which flower naturally much later and, therefore, are much larger are not particularly meaningful though such comparisons are fairly common.^{55,98-100}

Variation in susceptibility of pineapple to forcing agents is observed even after plants are large enough to produce a marketable fruit. In some cases, larger plants were easier to force than smaller ones,^{23,25,33} but very large plants are also reported to be difficult to force.^{14,46,97,99,127} In many cases, the effects of plant size were evaluated by planting at one time followed by sequential forcing. The forcing results, thus, were confounded with changes in environment which could also influence plant susceptibility.

Pineapple plants are more susceptible to forcing near the time for natural inflorescence initiation and this is especially evident when NAA is used.^{25,30,31,63,69,75,99,107,113,155} Plants well supplied with water, having a high nitrogen content, and growing vigorously are more difficult to force than those that are mildly stressed for nitrogen.^{2,14,41,45,72,107,109,113} Based on data of Guyot and Py⁷² for Smooth Cayenne, percent nitrogen on a fresh weight basis in the basal white tissue of the youngest physiologically mature leaf (termed the "D" leaf)^{84,108,131} should be below 1.6% with good forcing being obtained when the value was 1.3%. It is usually recommended that no nitrogen be applied for 2 to 3 months prior to forcing, especially if difficulty in forcing is likely or if NAA is used.¹¹³

Forcing is more difficult in warm humid equatorial climates at most times of the year¹³⁹ and during the warmer seasons in other localities. Night temperatures of 25°C or greater reduced the susceptibility of Smooth Cayenne to forcing with ethephon.^{11,23} When shelter

temperatures exceed 30°C during the day forcing is not recommended, as forcing percentages obtained with ethephon⁴⁹ and NAA⁵⁰ declined approximately linearly with increasing temperature. There is need for additional research on the effects of day and night temperature on forcing success to improve the understanding of how the plant and the environment interact.

Differences in susceptibility between cultivars exist; a greater quantity of growth regulator was required to force Smooth Cayenne and Cabezona than was required for Red and Singapore Spanish.^{53,144,153}

4. *Effects of Forcing on Fruit and Propagule Production*

Forcing has little or no direct effect on fruit quality although fruit which develops during cold or cloudy periods will have reduced sugar and increased acid levels. Fruit shape and cannery recovery can be affected by forcing. NAA forcing resulted in a slightly larger fruit than that produced by forcing with other growth regulators,^{69,155} but generally the fruit is more conical^{112,123} so slice recovery may actually be reduced. Forcing with NAA also increases peduncle length^{112,113} which increases susceptibility to lodging but, because of better fruit exposure, may also be responsible for the reduced incidence of fruit disease observed in comparative studies with ethylene.¹²³ The use of more than 40 mg of ethephon per plant can result in fruit that are much shorter than normal.^{30,50}

The production of slips is consistently reduced with the use of NAA,^{75,112} but data for ethephon are inconsistent. Some workers report reduced slip numbers when compared to the control,^{14,53,77,99,100} while others report no effect.²⁴ The effect is less pronounced on large plants than smaller ones^{34,100} and control plants for all the above studies were larger and flowered later than those forced with ethephon. The obvious conclusion is that the effect of ethephon on slip numbers is due primarily to the fact that forced plants are smaller at the time of flowering than those that flower naturally.

5. *Forcing as a Commercial Practice*

Though few data are available, ethylene generally is reported to be a more effective forcing agent than acetylene, dry calcium carbide, ethephon, or NAA, especially in warm, humid climates.¹³⁹ Ethylene is applied at night at a rate of about 800 g/ha in 7000 ℓ of water containing 0.5% activated charcoal in West Africa.³⁷ It is also used on one plantation in Hawaii but no details are available on the specific quantities of material used. Special techniques are required for its application³⁷ so the use of ethylene generally has been restricted to larger plantations.

Water saturated with acetylene is used commercially by smaller growers in West Africa¹³⁹ where the scale of the enterprise is not large enough to justify the purchase of sophisticated spray equipment. The recommended rates in Reunion and West Africa are to add 200 g of calcium carbide to 75 or 100 ℓ of cool water and apply 50 to 80 ml of solution in the center of the plant.^{6,139} Py and Tisseau¹¹² reported that the percentage of plants forced increased progressively as water temperature was reduced from 25 to 7°C. However, Aldrich and Nakasone² consistently obtained higher forcing with water at 25 to 26°C than was obtained at 3 to 5°C. They speculated that reduced forcing resulted from too slow a rate of acetylene evolution from the cold water solution. A second application is often made 2 to 5 days after the first to increase the forcing percentage.^{2,43,44,75,98-100,113,139} The procedure and precautions for mixing calcium carbide with water have been described in detail.^{26,87,113}

Dry calcium carbide has also been used but it can be less effective than acetylene in solution^{2,26} and may cause plant injury.^{2,26,139} The details on the use of dry carbide in the literature are often sketchy with amounts often given in qualitative terms. In Taiwan 0.5 to 0.7 g of calcium carbide is placed in the center of the plant;⁷⁵ in South Africa a 7-mm pebble of calcium carbide is used³⁰ while in Cuba 2.0 g per plant was applied.⁵⁵ If there is no water