#### **Dr Robert Geneve** Auxin use in cutting propagation - Then and Now

Dr. Robert Geneve has been a faculty member of the University of Kentucky, Department of Horticulture for over 35 years performing teaching and research in seed biology and clonal plant propagation. With over 100 journal, proceeding and industry publications, and having serviced as a Past -President for the International Plant Propagators' Society – Eastern region, he is currently the

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### Auxin use in cutting propagation - then and now



Robert Geneve University of Kentucky



### Auxin use in cutting propagation - then and now

Brief history of auxin

Auxin delivery systems

Future directions





The greenhouse and nursery industry have seen



Colorful foliage annuals are in vogue.



#### Interspecific hybrids

Interspecific hybrids in geranium between *Pelargonium xhortorum* and *Pelargonium peltatum*.

They are triploid and therefore do not produce seed – no need for deadheading.



Interspecific hybrids

Hybrid spider flower (*Cleome*) are compact growing under two feet tall, are odorless, thornless and seedless.



x*Petchoa* is an Intergeneric hybrid betwen *Petunia* and *Calibrachoa*.

#### Intergeneric hybrids



Hormone theory and organ formation

Julius Sachs in 1880 postulated the existence of plant morphogens.

Root forming – Rhizocaline Flower forming – Florigen Stem forming - Caulocaline



Brief history of auxin discovery

1933 – Kögl *et al*. isolated auxin from human urine.

1934 – Kögl *et al*. identified compound as indole-3-acetic acid.

Über ein neues Auxin ("Hetero-auxin") aus Harn. 11. Mitteilung über pflanzliche Wachstumsstoffe<sup>1</sup>.) Von Fritz Kögl, A. J. Haagen-Smit und Hanni Erxleben. (Aus dem Organisch-chemischen Institut der Rijksuniversität Utrecht.) (Der Schriftleitung zugegangen am 8. August 1934.)

### Brief history of auxin discovery

1934 – Went developed a rooting bioassay using etiolated pea seedlings.

1934 – Went and Thimann test root forming capacity of various leaf extracts in pea bioassay.







Kenneth Thimann

1935 - Researchers at the <u>Boyce Thompson Institute</u> become very active in auxin and rooting in cuttings.



William Cooper was first Superintendant for Boyce Thompson Institute

The first report of IAA being used to stimulate rooting in cuttings was by William Cooper in 1935. He treated cuttings with IAA in <u>lanolin paste</u>.





Cooper, W.A. 1935. Hormones in relation to root formation on stem cuttings. Plant Physiology 10:789-794.

1935 – Synthetic auxins are created at the Boyce Thompson's Institute.

The synthetic auxins included the familiar indolebutyric acid (IBA) and α–naphthaleneacetic acid (NAA) compounds used by modern propagators.

Synthetic auxins showed root promotion in cuttings that was as good or better than IAA.

Zimmerman, P.W. and Hitchcock, A.E. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contr. Boyce Thompson Institute 7:209-229.



In 1936, the <u>soak method</u> replaced lanolin paste for auxin delivery.

Auxin was made soluble in alcohol and diluted in water.

Typical soak durations were 10 to 24 hours.

Hitchcock, A.E. and P.W. Zimmerman.1936. Effect of growth substances onthe rooting response of cuttings. Contr.Boyce Thompson Institute 8:63-79.



By 1937, researchers at the Boyce Thompson Institute showed that <u>auxin stimulated</u> <u>rooting in over 85 genera</u>, including woody plants that had proven too difficult-topropagate in the past.



Soon after, auxin "dust" formulations became available.

In 1938, Chadwick and Kiplinger treated a variety of cuttings with IBA in a soak solution or as commercially available dusts.

They treated over 100 types of woody cuttings.

Interestingly, they reported IBA sprays were not as effective as basal soaks.

Chadwick, L.C. and D.C. Kiplinger, 1938. The effect of synthetic growth substances on the rooting and subsequent growth of ornamental plants. Proc. Amer. Soc. Hort. Sci. 36:809-816.



Lewis Charles (L.C.) Chadwick

The <u>Boyce Thompson Institute</u> was granted a patent for use of auxins in rooting and subsequently licensed Merck to distribute Hormodin A for commercial application.

By 1947, four commercial companies were offering synthetic auxin formulations in talc for application to cuttings.

Hormodin – Merck

Rootone - American Chemical Paint Co.

StimRoot - Plant Products Co.

Quick-Root - Dow Chemical,

October, 1940

Research Bulletin 280

Effect of Hormodin A, a Growth Substance, on the Rooting of Cuttings



#### Commercial auxin adoption

Propagation of Trees, Shrubs and Conifers Wilfrid G. Sheat London, 1948.

"I think it is true to say that to date no real commercial advantage has yet been gained by the use of the substance (auxin) for the production of plants by cuttings."

"As a practical propagator, I would add a word of warning. The use of chemical root-producing materials is no substitute for the exercise of intelligent practice in the art of propagation."



#### Commercial auxin adoption

James Wells. 1951. Propagating Rhododendrons from stem cuttings. Proc. IPPS 1:12-14.



"HORMONE TREATMENTS – There are growers who say that there are no results obtained by the use of hormones which the skilled propagator cannot develop without them."



#### Commercial auxin adoption

James Wells. 1951. Propagating Rhododendrons from stem cuttings. Proc. IPPS 1:12-14.



"This is an argument to which we do not subscribe. We believe that used intelligently the plant hormones have a most definite place in modern plant propagation and we use them extensively."

"For our easily rooted varieties we use a powder containing 6 mg/g of indole butyric acid. This is the strongest commercially available powder in this country."

#### Commercial auxin adoption

Richard Fillmore. 1951. General review of woody plant propagation. Proc. IPPS 1:40-50.



"The use of synthetic hormones is a well established and often beneficial practice in rooting cuttings."

"Assuming that one is thoroughly familiar with the most suitable hormone and the optimum concentration for the species under consideration, hormone treatments will unquestionably promote improved results with a wide variety of plants."

#### Commercial auxin adoption

Richard Fillmore. 1951. General review of woody plant propagation. Proc. IPPS 1:40-50.



"When the requirements of this assumption cannot be met, the indiscriminate use of hormones may do more to inhibit than to promote rooting."

"I do not wish to be misunderstood. I am a pro-hormone man and I have successfully used hormones on dozens if not hundreds of species."

#### Commercial auxin adoption

John Vermeulen. 1954. The propagation of *Taxus* by cuttings. Proc. IPPS 4:76-81.



"The use of hormones! You know, I never went to high school. I never went to college, and I am awfully dumb in chemicals."

"I am quite thick-headed as a Dutchman and I didn't want to try a lot of things."

"I probably should have, but we finally got to the point we are trying chemicals."

#### Commercial auxin adoption

John Vermeulen. 1954. The propagation of *Taxus* by cuttings. Proc. IPPS 4:76-81.



"This year for the first time, we tried some indolebutyric acid, one and two percent. I don't know whether it will work out."

"I am not alone down there anymore, so they tell me I shouldn't be so old-fashioned, I should try some new things."

"We have to give in once in a while to the younger generation.

So we have!"

Commercial auxin adoption

**IPPS Proceedings Vol. 9 1959** 



Charles E. Hess

#### A COMPARISON BETWEEN THE QUICK DIP AND POWDER METHODS OF GROWTH SUBSTANCE APPLICATION TO CUTTINGS

CHARLES E. HESS Purdue University Lafayette, Indiana

There were seven papers in Volume 9 about the "quick dip" method.

#### A STUDY OF PLANT GROWTH SUBSTANCES IN EASY AND DIFFICULT-TO-ROOT CUTTINGS

CHARLES E. HESS Purdue University Lafayette, Indiana

Plant Propagation: Principles and Practices Hartman and Kester 1959

There are several groups of substances considered plant hormones.

These are (a) auxin, (b) traumatic acid, (c) caulines, and (e) vitamins.

Auxin appears to act as a sort of "master hormone".



Dale Kester

### Auxin application methods

- Dilute soak
- Quick dip
- Talc
- Foliar spray
- Total immersion



The <u>dilute soak method</u> is an older procedure where the basal part of the cutting is placed in the auxin solution.

Timing – up to 24 hours.



The <u>dilute soak method</u> is an older procedure where the basal part of the cutting is placed in the auxin solution.

Timing – up to 24 hours.

<u>Concentration</u> – 50 to 150 ppm.

<u>Solution type</u> – must be in water.

<u>Effectiveness</u> – works well but is cumbersome because of timing.



The <u>quick dip method</u> is a common treatment. The base of the cutting is immersed in the solution.

<u>Timing</u> – 3 to 5 seconds.





The <u>quick dip method</u> is a common treatment. The base of the cutting is immersed in the solution.

Timing – 3 to 5 seconds.

<u>Concentration</u> – 500 to 10,000 ppm.

<u>Solution type</u> – up to 50% solvent (ethanol). Potassium salts in water.

<u>Effectiveness</u> – very effective and uniform way to treat cuttings. Some cuttings may be sensitive to solvent.



The <u>talc (powder) method</u> is also a common treatment.

The base of the cutting is coated with the auxin talc.





The talc (powder) method

<u>Concentration</u> – 0.1 to 8.0%.

<u>Carrier</u> – talc is the most common.

<u>Effectiveness</u> – an effective treatment, especially for herbaceous cuttings.

A pre-dip in a solvent (water or ethanol) may increase effectiveness.



The <u>foliar spray method</u> uses an atomizer to spray the foliage.

<u>Timing</u> – until the solution drips from the leaves.



The <u>foliar spray method</u> uses an atomizer to spray the foliage.

<u>Timing</u> – until the solution drips from the leaves. It is applied after sticking.

<u>Concentration</u> – 100 to 5,000 ppm.

<u>Solution type</u> – usually the potassium salt of IBA in water.

<u>Effectiveness</u> – varies by species, but when effective it reduces worker exposure and labor time.


	Auxin and rooting in mum cuttings	1000
Treatment	Roots per cutting	
Untreated	19.7	



Auxin and rooting in mum cuttings		
Treatment	Roots per cutting	
Untreated 19.7		
Talc	29.5	



Auxin and rooting in mum cuttings		
Treatment	Duration	Roots per cutting
Untreated		19.7
Talc		29.5
Quick dip	1 seconds	41.3
	3 seconds	40.5



Auxin and rooting in mum cuttings		
Treatment	Duration	Roots per cutting
Untreated		19.7
Talc		29.5
Quick dip	1 seconds	41.3
	3 seconds	40.5
Dilute soak	8 hours	40.4
	16 hours	42.0



Auxin and rooting in mum cuttings		
Treatment	Duration	Roots per cutting
Untreated		19.7
Talc		29.5
Quick dip	1 seconds	41.3
	3 seconds	40.5
Dilute soak	8 hours	40.4
	16 hours	42.0
Spray		38.2



The <u>total immersion method</u> submerges cuttings in K-IBA solution.

<u>Timing</u> – several minutes.

<u>Concentration</u> – 100 to 5,000 ppm.

<u>Solution type</u> – usually the potassium salt of IBA in water.

<u>Effectiveness</u> – varies by species, but is time efficient (batch treatment).



Total immersion method - background

Stock plant specialists and shipping unrooted cuttings.





### Total immersion method - background

Reinforced awareness of importance of hydrating cuttings.

Use of surfactants.



## Total immersion method - background

Sanitation including disinfectants in washing solution.



### **Total immersion method - background**

Biopesticides treatment of cuttings.



### Total immersion method



Stacking immersion solutions

UpTake = wetting agent

Suffoil-X = mineral oil

K-IBA at 1000 ppm

### **Total immersion method**



### Foliar spray

Effective foliar auxin may be impacted by:

Species type

Auxin uptake, transport or metabolism

Timing of spray application



### Foliar spray

Roots per cutting in *Euonymus alatus* cuttings with auxin applied as a quick dip or to the abaxial (upper) or adaxial (lower) leaf surfaces with or without a surfactant.





No difference in roots per cutting between adaxial vs. abaxial auxin application.

### Foliar spray

Roots per cutting in *Euonymus alatus* cuttings with auxin applied as a quick dip or to the abaxial (upper) or adaxial (lower) leaf surfaces with or without a surfactant.





Rooting percentages were lower in adaxial treated cuttings and this was recovered using the surfactant.

Foliar spray

Comparison of stomatal density in (a) abaxial and (b) adaxial leaf surfaces in *Euonymus alatus*.

Adaxial (upper surface) ~ 15 stomata

Abaxial (lower surface) >100 stomata



#### Foliar spray

Roots per cutting in *Cephlalanthus* cuttings with auxin applied as a quick dip or to the abaxial (upper) or adaxial (lower) leaf surfaces.



## Foliar spray

ranaea paniculata

Impact of application timing and multiple applications on rooting.

### Foliar spray

Roots per cutting in *Hydrangea paniculata* 'Limelight' cuttings with auxin applied as a quick dip or as single or multiple foliar sprays on 2, 4, or 6 days after sticking.



#### Foliar spray

Roots per cutting in *Hydrangea paniculata* 'Limelight' cuttings with auxin applied as a quick dip or as single or multiple foliar sprays on 2, 4, or 6 days after sticking.



Foliar spray was more effective than quick dip.

Roots per cutting for auxin increased when applied at six days after sticking.

Multiple sprays generally showed an increase in roots per cutting over single sprays.

### Foliar spray

Roots per cutting in *Viburnum dentatum* cuttings with auxin applied as a quick dip or as single or multiple foliar sprays on 2, 4, or 6 days after sticking.





## Foliar spray

Roots per cutting in *Viburnum dentatum* cuttings with auxin applied as a quick dip or as single or multiple foliar sprays on 2, 4, or 6 days after sticking.



Foliar spray was as effective as quick dip.

No difference in roots per cutting for auxin applied up to six days after sticking.

Multiple sprays showed an increase of about 10 roots per cutting.

## Foliar spray

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Foliar spray was as effective as quick dip.

No difference in roots per cutting for auxin applied up to six days after sticking.

Multiple sprays showed an increase of about 10 roots per cutting.

No difference in rooting percentage.

## Foliar spray

Including growth regulators in the K-IBA foliar spray.





#### Foliar spray

Root formation in *Angelonia* cuttings treated with K-IBA and Bonzi as a dip or foliar spray.

Application method	K-IBA (mg · L <sup>-1</sup> )	Bonzi (mg · L⁻¹)	Rooting percentage	Roots per cutting
Untreated	0	0	79.7c	3.9d
Foliar spray	0	20	➡ 95.8b	➡ 6.1c
	100	0	100a	5.9c
	100	20	100a	7.1c

#### Foliar spray

Root formation in Angelonia cuttings treated with K-IBA and Bonzi as a dip or foliar spray.

Application method	K-IBA (mg · L <sup>-1</sup> )	Bonzi (mg · L <sup>-1</sup> )	Height (cm)
Untreated	0	0	6.2
Quick dip	1000	0	7.5
	1000	20	7.7
Foliar spray	0 100 100	20 0 20	7.3 7.5 7.7

Foliar spray

Root formation in Angelonia cuttings treated with K-IBA and Bonzi as a dip or foliar spray.



#### Foliar spray

Some species do not have the same response to foliar auxin compared to basal dips.

Species	K-IBA	Rooting percentage	Roots per cutting
	Untreated	85	6.4
Euonymus	Quick dip	98	<b>13.3</b>
	Foliar spray	89	9.2

#### Auxin transport

In Arabidopsis hypocotyls, applied IBA and IAA moved basipetally at different speeds.

Significant amounts of IBA were converted to IAA during transport.

Conjugated IBA accumulated at the base preferentially to active free IBA.



#### Auxin transport

Can we manage polar auxin transport?

For example, dopamine inhibits oxidation of IAA and enhances auxin transport at AUX1.



### Combining auxin with other growth substances

Impact of foliar applied benzyladenine on shoot regrowth in Rudy Haag euonymus.



Combining auxin with other growth substances

The influence of adenine and benzyladenine on rooting and development of *Fuchsia hybrida* cuttings.

Species	Rooting percentage	Roots per cutting
Untreated	65	5.0
NAA + BA	➡ 64	➡ 0.5
NAA + Adenine	➡ 87	9.0

K. Wróblewska. 2012. The influence of adenine and benzyladenine on rooting and development of *Fuchsia hybrida* cuttings. Acta Agrobotanica 65: 101–108.

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Hormone theory and organ formation

Julius Sachs in 1880 postulated the existence of plant morphogens.

Root forming – Rhizocaline Flower forming – Florigen Stem forming - Caulocaline



### Rhizocaline

**Rhizocaline** is a term related to plant biology. It refers to a hormone or hormonelike factor that is distinct from auxin (another plant hormone).

Rhizocaline is believed to play a role in the formation of plant roots. However, its exact mode of action remains somewhat mysterious.

In summary, rhizocaline is an intriguing component in the complex world of plant growth and rooting processes.



## Graft transmissible substances (Rhizocaline)

There are graft transmissible substances that move from leaves to the base of a cutting that improve rooting.

MicroRNAs including miR156 are found to control vegetative phase transition by regulating phase change genes.

miR156 is graft-transmissible.

Reciprocal grafts between juvenile and mature English ivy.


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