Presented at the International Plant Propagator's Society, Eastern Regional Meeting, October 17, 2009

# **Propagation of Plants from Cuttings Using Rooting Solutions by Foliar Methods**

# Joel Kroin

Hortus USA Corp., PO Box 1956 Old Chelsea Sta., New York NY 10113 support@hortus.com

The natural plant hormone, IAA, an auxin, is bio-synthesized in plant leaves APPLIED AUXINS (IBA, NAA, or IAA) ENTER THE PLANT THROUGH OPEN STOMATA Using water based solutions, auxins (IBA, NAA, or IAA) are applied to the eaves. The auxins are absorbed into the vascular system through open stomata. The stomata are open when TRANSLOCATION the plant is not under heat or cold THROUGH stress or dehydration. The natural and applied auxins translocate from the leaves to the sink point, the basal end of the cutting. They move by mass flow, cell by cell, in a polar manner. STORAGE AND USE OF Auxins are stored at the basal end of the cutting. The plant self regulates the AUXINS FOR ROOT need for the auxins. The reason for FORMATION auxins effecting root formation is not known. Possibly the applied auxin (IBA)

ROUTE OF FOLIAR APPLIED ROOTING SOLUTIONS THAT CONTAIN AUXIN FOR USE IN ROOT FORMATION

is converted to the natural auxin, IAA,

and used for root initiation and growth.

FIGURE 1 All graphics by Hortus USA

AUXIN

MASS

FLOW



# INTRODUCTION

I have never completely read a whole book on plant propagation nor a complete chapter. I did however read a popular book on nursery management. W ritten in '96, the esteemed writer explains that the best way to propagate plants is to use it's natural reproduction ability. Perhaps you own this popular book, the Nursery Book, written by Liberty Hyde Bailey, not 1996 but 1896, more than 120 years ago. He did not discuss plant rooting substances since scientists had not yet identified them (Bailey, 1896).

Contemporaries of Bailey, Darwin and other scientists, identified the polar transport of natural substances from the apical part of the plant downward. Plant researchers had long known that plants produce substances that cause dormant cells to divide and become roots. In 1934, Thimann and Went identified the substance to be IAA (Indole-3-acetic acid), a plant growth regulator, now called an auxin. Scientists soon proved the ideas of Darwin. Plants produce auxins in leaves (Thimann, 1977) (Darwin, 1880). The auxins move from the apical to the basal part of plant cuttings. Foliar application of bio-simulators of the natural auxin travel like the natural auxin. When used for root initiation, the threshold amount of all auxins are accumulated and utilized at the basal end.

## **AUXIN METABOLISM**

The natural auxin IAA, produced during the development of leaves, is found in 'free' and 'bound' states. 'Free auxins' are available immediately. They move within the plant in polar transport, from the elongating leaf tips and continue downward, through the vascular system, to the basal end (Aloni, 2004). Free auxins are present when auxins are dissolved in water to make 'rooting solutions'. 'Bound auxins' are variable and limited in their ability as plant growth regulators. Bound auxins are present when auxins are made into dry powder 'rooting hormones' and blended with lanolin. Bound compounds, applied close to the basal end, do not need polar transport to initiate roots since they are close to where roots are to be formed (Leopold, 1955, pgs. 66-7). FIGURE 1

# **BIO-SIMULATORS OF THE NATURAL AUXIN**

The natural auxin, IAA is unstable and degrades rapidly in the presence of light and heat. More stable than IAA, the bio-simulators IBA (Indole-3-butyric acid) and NAA (Naphthalene acetic acid) are commercially available. They remain active for use by plants over a long time. They are regulated by the plant for many purposes, including inducing root formation. Scientists do not fully understand the way that plants use auxins to induce root formation. As of 2009, as single component auxin, only IBA is US EPA registered. In Europe all three are registered in several countries (NOTE 1). Of the three, IBA is the most useful auxin to propagate plants from cuttings by inducing root formation. For plant propagation, several basal and foliar auxin application methods are used. The methods and rates depend upon plant varieties, juvenility, season and other factors.(Rhizopon, 2004).

# POLAR TRANSPORT

Produced in leaves, plants transport IAA, and other auxins, cell to cell, to the basal end. The plant regulates the speed of motion based upon physiological factors, such as water status. Relative rates of movement are: IAA @ 7.5 mm/hr., NAA @ 6.7 mm/hr., and IBA @ 3.2 mm/hr. The rate of flow is not critical since auxin use is slow (Epstein, 1993). As the auxins travel they accumulate in higher concentrations at the basal end (Thimann, 1977). **FIGURE 2** 

Two physiologically distinct and spatially separated pathways function to transport auxins over long distances through plants, the polar and non-polar routes. FIGURE 3. In the 'polar route', auxin is translocated by mass flow and other metabolites in the mature phloem. Transport is downward from immature tissues close to the shoot apex toward the root tips. In aqueous solution, free auxin, loaded into a mature phloem, is translocated passively in the phloem sap to sink organs and tissues at the basal end where it is released (Aloni, 2004). Free auxin moves in the primary shoot through the epidermis, bundle sheath, vascular meristem, and xylem. In the secondary body through the phellogen and cambium. In the primary root through the epidermis, pericycle, and vascular meristem. In the 'nonpolar route', auxins move up and down the sieve tubes (Aloni, 2004). Upward flow is apparent when there is an excess of auxins beyond the threshold level needed by the plants, sometimes causing aerial roots to form (Thimann, 1977).

# AUXIN METABOLISM AT THE BASAL END

IBA was shown to be metabolized by the plant to IAA in a slow release process, thereby



# Free Auxin Transport in Plant Shoot, Body and Primary Root





BASAL END OF CUTTING, STORAGE POINT OF AUXINS: Metabolic conversion of IAA <> IBA available to be utilized by plant tissues for root formation Simplified IAA and IBA metabolism links as suggested by Epstein and Ludwig-Muller, 1993. Indole-3-butyric acid in plants, occurence, synthesis, metabolism and transport. Physiologia Plantarum 88:382-9 FIGURE 4

# CONTEMPORARY FOLIAR METHODS

In 1985, Kees Eigenraam was the technical advisor for Rhizopon bv in Holland. He knew Rhizopon auxin rooting products, made into aqueous solutions, regulate fruit and flower drop when applied to the leaves of plants. He found that **auxin solutions applied to leaves had a positive effect on root initiation**. He did trials at Dutch greenhouses where he developed two methods of application.

Using the **Total Immerse Method**, cuttings are totally immersed in the auxin rooting solution for five seconds, then stuck in media. The cuttings require no further treatment. Dipping is usually done in a basket, with a few cuttings to avoid breakage. This method is especially useful for very small production lots. It is also good for

allowing steady use of the unstable IAA. Stable IBA was found to be an endogenous, synthesized, constituent of various plants. Like IAA, IBA is transported mostly in 'basipetal direction polar transport', from apex to the base. Research by Epstein found that the plants studied were able to hydrolyze auxin conjugates during growth to time release 'free auxin' which may induce root initiation (Epstein, 1993). This theory is supported by Epstein's reports on increased level of free auxin in the bases of cuttings prior to rooting. Studied in grape and olive, the plants were shown to convert IBA to IAA, where, IAA was shown to accumulate at the basal end. The higher rooting promotion of IBA was also ascribed to it's stability relative to IAA which is short lived, though, IBA was metabolized and used up in plant tissue (Epstein, 1993, 1984). FIGURE 4

# AUXIN EFFECTS ON PLANTS (Based upon Davies, 2004)

# Root formation effects of auxins:

- cell enlargement (increase root and stem length).
- cell division (assists in root formation).
- root initiation (induces roots on stems and sometimes leaves).
- apical dominance (effects the stem and leaf growth when using foliar applied auxins).
  - tropic responses, bending (sometimes noticed on tender leaves when using foliar applied auxins).
- Other plant growth effects of auxins:
  - leaf senescence (delay of leaf drop).
  - leaf and fruit abscission (leaf and fruit drop).
  - fruit setting and growth.
  - promotes flowering in some plants like bromeliads
  - growth of flower parts.
  - In some cases, the effect of excess auxins is to inhibit growth.

# EARLY AUXIN APPLICATION METHODS

From the early 1930's, researchers applied auxins with:

#### Auxin dry dip powder compounds

Method: applied to the basal end of the cuttings.

Formulation: powders were made with auxins blended with talcum powder or powdered charcoal. • Auxin rooting solutions

• Auxin rooting solutions

Method: applied to the basal end of cuttings by basal long soaks Formulation: early auxin rooting solutions were made in low concentration using alcohol as carrier. After 1939, tablets made by Rhizopon, in Holland, allowed auxin solutions to be made using water. These solutions were used for plant rooting and other plant growth regulation operations.

Auxin lanolin paste

Method: applied to the basal end and also to plant leaves (Mitchell, 1947) (Thimann, 1937). Formulation: pastes were made with lanolin from wool blended with auxins

# EARLY FOLIAR OBSERVATIONS

In 1946, van Overbeek observed that the action of the natural plant rooting hormone in the leaves of plants is essential for plant cuttings to form roots at the basal ends. The rooting hormones move from the leaves to the basal end where they are stored (van Overbeek, 1947). Thimann and W ent, in their 1937 book Phytohormones, discuss trials where they applied lanolin-auxin compounds to various parts of the plant. Application to the apex and also the basal end both had positive rooting effects. *"When auxin is applied to the apex, the lowest concentration needed to produce localized roots in this way is about 100 times that needed to produce roots at the base"* (Thimann, 1937, pg. 201). Using viscous lanolin-auxin compounds they had 'bound auxins' which had difficulty translocating to the basal end. Therefore, they needed high rates by apex application.

# THE LEAF STOMATA AND ENTRY OF AUXINS INTO THE VASCULAR SYSTEM

Leaves have **stomata**, pores that allow the plant to transpire gases, oxygen and carbon dioxide, and liquids. The stomata are protected, each by two guard cells. These cells cause the stomata to be open during normal room temperature and close in heat or cold. Under the guard cells are air spaces. Aqueous auxin solutions contain 'free auxins'. The solutions when applied to leaves, enter open stomata and are entrapped in the air space. After entry, free auxins can flow through the vascular system (Leopold, 1955, pgs. 791-2). FIGURE 5

large homogenous lots taken from a large parent stock. Since the cuttings drag in biologicals, to avoid cross contamination of pathogens, the rooting solution should be disposed after four to five hours of use, or minimum daily (Rhizopon, 2004) (Kroin, 2009).

Using the **Spray Drip Down Method** the cuttings are first stuck in media. The rooting solution is sprayed onto the cuttings until the liquid drips off the leaves. Minimum labor skills are needed. No PPE is required since cuttings are untreated. Tank mix sprayers are used (hand, backpack or hydraulic). Boom sprayers do not provide good control and proportional mixers give inconsistent mixing. One skilled operator can treat large production areas in a few minutes. Misters can be turned on after the rooting solution dries on the leaves or 30 to 45 minutes. In hot climates spraying is done early in the morning because stomata are open and accept the solution. The solution is used one time; there is no cross-contamination between crops due to the treatment (Rhizopon, 2004).

The Spray Drip Down and Total Immerse Methods are labor saving since the workers to not individually treat the cuttings.

# YODER BROTHERS & BAILEY NURSERIES ADOPT FOLIAR **METHODS**

In 1994, Kees and I visited Lyraflor in Holland, one of the world's largest chrysanthemum rooting stations. Lyraflor used robotics. The robots placed the trays of cuttings in the propagation house. They then sprayed the rooting solutions on the cuttings. We also visited pot rose and hedera (ivy) growers who totally immersed the cuttings then stuck (Kroin, 2009). These growers all used Rhizopon AA Water Soluble Tablets containing IBA.

In the US, Kees and I visited the Yoder Brothers chrysanthemum Florida stock plant facilities (now Syngenta Flowers). At that time they used rooting solutions by basal quick dip. We introduced them to foliar application. Water soluble IBA products were selected because they are US EPA registered for use by plant growers. Soon after their Yoder-Greenleaf Perennials growers (now Aris Horticulture, Green Leaf Plants div.) did foliar trials on their perennials. They adopted the Spray Drip Down Method using Hortus IBA Water FIGURE 5 Soluble Salts (Yoder, 2004, 2008-9)

A few years later Sam Drahn at Bailey Nurseries developed a program using the Spray Drip Down Method on woody ornamental plant cuttings using Hortus IBA Water Soluble Salts (Drahn, 2003, 2007). The Bailey trials were innovative since the technique had been previously used mostly on annual and perennial cutting. Sam began his trials with high concentrations of rooting solutions made with the Salts (Drahn, 2003, 2007). Later he found that lower rates also achieved good rooting. (Drahn, correspondence)

Yoder, Aris, and Bailey developed spray methods suited for their operations. Both companies do sticking all day and spray the next work day, even after a weekend. The Yoder-Greenleaf Perennials, Lancaster, PA facility, made a spray cart to allow spraying on either side of the aisles. Bailey Nurseries plant their cuttings in beds or pots; they spray the cuttings using a hydraulic sprayer.

# FOLIAR RATES (refer to charts)

# **Perennial and Woody Ornamental Plants**

While Yoder, Aris, produce perennials and Bailey produce woody ornamental plants, they each started trials at relatively high rates: 1500 to 2000 ppm IBA as Hortus IBA Water Soluble Salts. Over time they found satisfactory results using lower rates. Winter hardy perennial and woody ornamental plants have almost the same rates: perennial plants at 250 to 1500 ppm IBA, and woody ornamental plants at 350 to 1500 ppm IBA. Rates were established using Hortus IBA Water Soluble Salts (Yoder & Bailey correspondence) **Annual Plants** 

For cuttings from **annual plants**, those plants that are not hardy or short season, target rates are from **80 to 250 ppm IBA**. Higher rates may not cause permanent damage to the cuttings but they might get leaf curl or leaf spotting on the treated leaves. These effects are not permanent as the new leaf growth will be normal and there will be a high root mass. The effect is likely not caused by the rooting solution. Rather, the cuttings might have had inadequate stock plant preparation. Light and fertilization are needed before cuttings are taken to assure that they build up carbohydrates. Rates were established using Hortus IBA Water Soluble Salts Slow to Root Plants

Slow to root cuttings, planted in trays or pots, can be sprayed to improve root formation. Spray rates are similar to initial rates by the Spray Drip Down Method. To bring young rooted plants up to a uniform standard, Dutch growers use the spray drip down method at 50 to 100 ppm IBA, using Rhizopon AA Water Soluble tablets at 1 to 2 tablets/liter of water (Eigenraam, correspondence).

# LEAF CROSS SECTION



## WHEN AND WHEN NOT TO USE FOLIAR APPLICATION

Plants not suitable to be treated by foliar methods are leafless cuttings and winter dormant cuttings. Yoder and Bailey both found that about 15 percent of leafy cuttings in the growing season are not suitable to propagate by foliar methods. Suitability of plants is variable based upon reasons such as the season, variety, condition of the stock plant before taking cuttings, and storage conditions. Sometimes the plants are better propagated by basal methods such as the Basal Quick Dip, Basal Long Soak, and the Basal Dry Dip Methods (Kroin, 2009) (Yoder and Bailey Nurseries, correspondence).

# DISCUSSION

L. H. Bailey was correct to say, when propagating plants you must use the full potential of the plant itself. Due to lack of technology, in Bailey's time, propagation from cuttings was not widely performed, especially on woody plants. Whatever propagation method he suggested to use, his goal was to use a stock plant that "is vigorous, free from disease or blemishes, and that possesses the characteristics of that variety." These "first class" plants are well grown, mature, and of the proper age for propagation (Bailey, pg.142).

Kees Eigenraam says, "mother plants only produce easy-to-root cuttings when they are biologically and physiologically young" (Eigenraam, correspondence).

L. H. Bailey and Eigenraam are correct in their view to select the very best plants, at the proper time, to achieve successful propagation. Using their potential, plants produce the natural root forming substance IAA, auxin, in their leaves and other parts. Biosimulators of IAA are IBA and NAA. For propagation from cuttings, during the growing season, leafy plants can accept aqueous auxin rooting solutions through their leaves though pores called stomata. Aqueous rooting solutions contain free auxins that are active and can move within the plant. They move cell to cell, from the leaves to the basal end, where they accumulate. At the basal end the plant self regulates the use of the stored auxins for root initiation and other processes. The Spray Drip Down and Total Immerse Methods have been shown to be useful to apply these rooting solutions to leaves. When using foliar methods at the proper time, the plant uses it's ability to form new roots by utilizing natural and applied auxins.

#### REFERENCES

- Aloni, R. 2004. The Induction of Vascular Tissues. In Davies, P., Plant Hormones, Biosynthesis, Signal Transduction, Action! Kluwer Ach. Dordrecht, NL. Pg. 477.
- Bailey, L. H. 1896. The Nursery Book. Macmillan & Co., New York, NY. Davies P. J. 2004. Natural Occurrence and Functions, in Davies, P., Plant Hormones,
- Biosynthesis, Signal Transduction, Action! Kluwer Ach. Dordrecht, NL. Pgs. 5-6. Darwin, C. 1880. The Power of Movement in Plants. John Murray. London.
- Drahn, S. 2003. Replacing Manual Dips with Water Soluble IBA. Combined Proceedings of the International Plant Propagators' Society, Vol. 53, 2003, Pgs. 373-7.
- Drahn, S. 2007. Auxin Application via Foliar Sprays Combined Proceedings of the International Plant Propagators' Society, Vol. 57, Pgs. 274-7.
- Epstein, E. and Lavee, S. 1984. Conversion of Indole-3-butyric Acid to Indole-3-acetic Acid by Cuttings of Grapevine and Olive. Plant and Cell Physiology, Vol. 25, No. 5, Pgs. 697-703.
- Epstein, E. and Ludwig-Muller, J. 1993. Indole-3-acetic Acid in Plants: Occurrence, Synthesis, Metabolism and Transport. Physiolgia Plantarum, Vol 88, Iss.2, Pg 383-9.
- Friml, J., Zazimalova, E., and Morris, D. 2004. Auxin Transport, In Davies, P., Plant Hormones, Biosynthesis, Signal Transduction, Action! Kluwer Ach. Publ. Dordrecht NL. Pgs. 438-9.

- Kroin, J. 2009. Hortus Plant Propagation from Cuttings. A Guide to Using Plant Rooting Hormones by Foliar and Basal Methods. Hortus USA. New York NY.
- Kroin, J. 1992. Advances Using Indole-3-butyric Acid (IBA) Dissolved in Water for Rooting Cuttings, Transplanting and Grafting. Combined Proceedings of the International Plant Propagators' Society, Vol 42.
- Leopold, A.C. 1955. Auxin and Plant Growth. Univ. of California Press. Berkeley, CA. Mitchell, J.W., and Marth, P.C. 1947. Growth Regulators for Garden, Field and Orchard. Univ of Chicago Press. Chicago, IL. Pg. 34.

Rhizopon Rooting Guide. 2004. Rhizopon b.v., Hazerwoude, Holland.

- Thimann, K. V. and Went, F. 1934. On the Chemical Nature of the Root Forming Substances. Proc. Of the Royal Acad. Amsterdam, Netherlands. Vol. 37, Pgs. 457-9. Thimann, K. V. and Went, F. 1937. Phytohormones. 1st ed., Macmillan, New York, NY.
- Thimann, K. V. and Went, F. 1937. Phytohomotes. 1st ed., Macminan, New York, NT. Thimann, K. V. 1977. Hormone Action in the Whole Life of Plants. University of Massachusetts Press. Amherst, MA.
- Van Overbeek, J., Gordon, S., and Gregory, L. 1946. An Analysis of the Function of the Leaf in the Process of Root Formation in Cuttings. Am J of Bot, Vol. 33, Pgs. 100-7.
  Yoder Garden Mums and Asters. 2008-9. Yoder Brothers. Barberton, OH.
  Yoder Handling Un-Rooted Perennials. 2004. Yoder Brothers. Barberton. OH.

NOTE 1: Some international registrations as single component auxin rooting products: IBA: US (US EPA registered), IBA, NAA and IAA: Holland, UK, Ireland, Slovenia, Poland, Czech Rep. IBA: Slovenia. NAA: Ireland (Rhizopon correspondence)

# TRIAL RATES

Water soluble IBA products used to make rooting solutions were selected because they are US EPA registered for use by plant growers. Rates are based upon trials at many locations (including Rhizopon users, and Yoder, Aris, and Bailey). Individual results may vary; trials are needed.

TRIAL RATES FOR CUTTING TYPES	ppm IBA - Using Water Soluble IBA
Soft Perennial Cuttings, Annual Cuttings	80-250
Perennial Cuttings	250-1500
Woody Ornamental Cuttings	350-1500
	Rates can be lower for juvenile cuttings

Conversion for Hortus IBA Water Soluble Salts used by Yoder, Aris and Bailey Nurseries: 50 ppm as Water Soluble IBA= 1 Rhizopon AA Water Soluble Tablet /liter water = 250 mg Hortus IBA Water Soluble Salts /liter water.

# Typical ANNUAL PLANTS propagated by the Spray Drip Down & Total Immerse Methods (ppm IBA-using Water Soluble IBA)

Pelargonium geranium		peltatum	300-400	Petunia sp.	150-200	Verbena	200-300
sp. like <i>Balcon</i>	50-100	Impatient, New Guinea	15-50	some colors	200-300	Poinsettia	25-100
zonale	200-300	Fuchsia	15-50	Osteospermum	150-200		

## Typical PERENNIAL PLANTS propagated by the Spray Drip Down & Total Immerse Methods (ppm IBA-using Water Soluble IBA)

Abutilon	750	Coleonema	750	Iberis	1000	Rosmarinus	500
Achillea	up to 1000	Convolvulus	750	Itea Little Henry	1000	Rudbeckia	750
Actinidia Arctic Beauty	1000	Coreopsis	500-1000	Kerria	1000	Ruellia	1000
Ajuga	up to 1000	Correa	500	Lamiastrum Herman Pride	1000	Salvia	500-1000
Amsonia	1500	Cosmos	1000	Lamium	up to 1000	Santolina	500
Anisodontea Tara's Pink	750	Cotoneaster Coral Beauty	500	Lavandula	1000	Saponaria	1000
Antennaria	up to 750	Delosperma	1000	Leptospermum	500	Saxifraga	750
Anthemis	1000	Erigeron	750-1000	Linaria	500	Scabiosa	1000
Arabis Variegata	500	Erodium Dark Eyes	750	Lithodora	2000	Silene	500
Arctostaphylos	500	Erysimum	750	Lonicera	1000	Solly Boddy's Choice	750
Armeria	1000	Escallonia Compacta	500	Lychnis	1000	Spilanthes	500
Artemisia	up to 500	Eupatorium	500	Marjoram Compactum	500	Spiraea <i>sp</i>	1000
Baptisia	3500	Euphorbia	1000	Melissa	up to 500	Spiraea Gold Flame,	
Basil Kasar	500	Gaillardia	500	Mentha	500	Magic Carpet, Neon Flash	1000-2000
Buddleia	1000	Galium Sweet Woodruff	1500	Nepeta	500	Stachys	1000
Calamintha Variegata	500	Geranium	1000	Oenanthe	500	Stevia rebaudiana	500
Callicarpa	500	Geum <i>Rivale</i>	1000	Origanum	500-750	Teucrium	1000
Campanula	500-1000	Gypsophila Viette's Dwrf	1000	Paxistima Canbyi	1000	Verbascum	1000
Caryopteris	1000	Hedera	1000	Penstemon	500	Verbena	750
Ceanothus	500	Helenium	500	Persicaria	up to 1000	Vinca	1000
Ceratostigma	1500	Helianthemum	2000	Phlox	1000	Viola	1500
Chrysanthemum	500-1000	Helianthus	1000	Phygelius	750	Vitex	1000
Chrysogonum	750	Helichrysum	500-1000	Poinsettia	500-1000	Waldsteinia	1000
Cistus	750	Heliopsis	1000	Polemonium Bressingham I	Purple 1000	Weigela	1000
Clematis	1000	Hypericum	1000	Prunella Loveliness	750	Westringia	750
Clethra	1000	Hyssop Pink Delight	500				

# Typical WOODY ORNAMENTAL PLANTS propagated by the Spray Drip Down & Total Immerse Methods (ppm IBA-using Water Soluble IBA)

,							
Acer	1000-1500	Juniperus, horizontalis	1000-1500	Rosa, varieties	1000-1500	Thuja	up to 1500
Cotoneaster	500-750	Physocarpus, opulifolius	1000-1500	Spirea, Japonica	500-750	Viburnum	1000-1500
Diervilla, paniculata	500-750	Rhus	500-750	Syringa	500-750	Weigela	1000-1500
Hydrangea	500-750						

#### SOURCES:

Personal correspondence with Rhizopon, Yoder, Aris, Bailey and other growers Yoder Handling Un-rooted Perennials. 2004. Yoder Brothers. Barberton, OH. Rhizopon Rooting Guide. 2004. Rhizopon b.v., Hazerwoude, Holland.