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MAY 21 2002

May 17, 2002

National Organic Standards Board
c/o Toni Strother
1400 Independence Avenue, South West
Room 4008-S, Ag Stop 0268
Washington, D.C. 20250

Ref: Petition for the listing of 6-Benzyladenine on the USDA National List
of Allowed and Prohibited Substances

Dear Mrs. Strother,

Enclosed you will find our petition and supporting documentation for the review of 6-Benzyladenine as possible candidate to be added to the USDA National List of Allowed and Prohibited Substances, in particular, § 205.601 Synthetic substances allowed for use in organic crop production.

6-Benzyladenine is an EPA registered biochemical pesticide with confirmed natural occurrence as cytokinin plant growth regulator. It is formulated as active ingredient in for example Promalin, an alternative apple thinning and sizing product manufactured by Valent Biosciences Corporation. Other plant growth regulators, such as gibberellic acids, have been used by organic growers for many years and have allowed organic growers the benefits of modern crop management within the scope of the National Organic Program Rules.

6-Benzyladenine is an additional natural fit in the organic crop management system. Although the active ingredient has to be chemically manufactured, extraction from natural sources has not been discovered, I have included supporting literature to show it's natural occurrence as Cytokinin B.

Thank you very much for your time and effort and that of the National Organic Standards Board in reviewing our petition for the organic status of 6-benzyladenine. If you have any questions please do not hesitate to contact me by phone: (847)-968-4722 or e-mail: dirk.ave@valent.com.

Sincerely,

Dirk Avé
Project Manager
Valent BioSciences Corporation

MAY 21 2002

Petition for the Inclusion of 6-Benzyladenine on the National Organic Standards Board List of Approved Organic Substances

With this petition, Valent BioSciences Corporation is requesting the evaluation of 6-Benzyladenine, as synthetic natural occurring active ingredient of the end-use products Promalin and Accel, for inclusion on the list of allowed substances for use in organic crop production.

6-Benzyladenine as Active Ingredient in Promalin and Accel for Use as Crop Production Aid

1. The common name for the substance:

6-Benzyladenine belongs to the class of cytokinin type of plant growth regulators. The names 6-benzyladenine (6BA) and N6-benzyladenine are used throughout this petition.

ISO common name	No official ISO name: benzyladenine is used in the literature
Chemical name	1H-purin-6-amine, N-(phenylmethyl)-
IUPAC name	N^6-benzyladenine
Chemical Abstract name:	N-(phenylmethyl)-1H-purin-6-amine
Synonyms	Cytokinin B 6-(Benzylamino)purine Additional synonyms are listed in Appendix A: phys chem. section.

2. The manufacturer's name, address and telephone number.

Manufacturer/supplier of active ingredient.

Borregaard Synthesis
9 Opportunity Way
Newburyport, MA 01950
Phone: 978-462-5555

K.H.Sunny, Inc.
2400 Dallas Street
Houston, TX 77003
Phone: 713-236-0745

Formulated end-use products and holder of agricultural use registration.

Valent Biosciences Corporation
870 Technology Way, Suite 100

Libertyville, IL 60048

Contact: Dirk A. Avé or Mark Beach
Phone: 847-968-4722 or 4752
Fax: 847-968-4801
E-mail: dirk.ave@valent.com
E-mail: mark.beach@valent.com

3. The intended or current use of the substance:

6-Benzyladenine has been used by non-organic growers for many years as active ingredient in fruit sizing and thinning products in apple growing regions as a non-toxic, environmental safer alternative to carbaryl.

4. A list of the crop, livestock, or handling activities for which the substance will be used. If used for crops or livestock, the substance's rate and method of application must be described. If used for handling (including processing), the substance's mode-of-action must be described.

6-Benzyladenine together with gibberellins are the active ingredients in two plant growth regulator products, Promalin and Accel, made by Valent Biosciences Corporation. 6-Benzyladenine is classified by EPA as biochemical pesticide and its natural occurrence as cytokinin B has been shown recently.

Timely foliar applications of this plant growth regulator allows for better crop management due to its fruit thinning and sizing effects and the increase in lateral budbreak and shoot growth. Fruit thinning is common practice in the fruit growing industry although organic growers have to resort to costly manual labor in order to obtain the desired fruit density. Maximizing the crop production tools available for organic growers will allow them to lower produce prices while maintaining high profitability.

In Appendix A, product labels and technical use bulletins are provided as reference to crops and end-use product use.

6-Benzyladenine was registered by EPA in 1979, and as more data on it's mode of action became available the Agency classified 6-benzyladenine as a biochemical pesticide in 1990. As a result of the EPA re-registration efforts and a petition by the registrant at that time Abbott Laboratories, 6-benzyladenine received an exemption from tolerance in 1994 (40 CFR Part 180.1150).

The use rates with Promalin (with 1.8% 6-benzyladenine) are:

- on apples for branching 125 – 500 parts per million (ppm) of end-use product applied by foliar spray or from 5000 – 7500 ppm applied as latex paint spot treatments,
- on non-bearing pears and cherries for branching, the foliar spray can be from 250 – 1000 ppm of end-use product.

- On developing apple fruit for sizing, 25 – 50 ppm applied as spray.

The use rates for Accel as an apple thinner are:

- one or two applications of 50 – 75 ppm to small fruit (10 millimeter diameter or less).

5. **The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.**

6-Benzyladenine is obtained from Borregaard Synthesis and K.H. sunny, Inc. (see entry # 2). 6-Benzyladenine is a naturally occurring compound that can be chemically manufactured much more easily (similar in reason for the chemical synthesis of insect pheromones).

The basic process consists of reacting adenine with excess benzyl alcohol and sodium hydroxide. The materials are combined and heated. The benzylation of adenine is typically completed after 9 – 11 hours at 200 – 210 degrees C. The batch is sampled to determine the completion of the reaction. The excess solvent is recovered by vacuum distillation and the slurry containing 6-benzyladenine is extracted with methylene chloride, neutralized, filtered and washed with water and dried to obtain a 99-100% pure technical grade material. Solvent used in the process is completely recovered and used for subsequent batches.

End-user products, such as Promalin and Accel contain 1.8% of the technical grade 6-benzyladenine.

6. **A summary of any available previous reviews by state or private certification programs or other organizations of the petitioned substance.**

The product Promalin PGR containing 1.8% 6-Benzyladenine (or N-(phenylmethyl)-1H-purin-6-amine) and 1.8% Gibberellins A_4A_7 has been submitted for OMRI review on July 9, 2001. After providing OMRI with additional information on 6-benzyladenine biochemical pesticide status and natural occurrence on April 12, 2002, OMRI responded with the advise to petition the National Organic Program to include 6-benzyladenine as allowed synthetic material in organic production.

7. **Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.**

6-Benzyladenine was registered by EPA in 1979, and as more data on its mode of action became available the Agency classified 6-benzyladenine as a biochemical pesticide in 1990. As a result of the EPA re-registration (RED) efforts and a petition by the registrant, 6-benzyladenine received an exemption from tolerance in 1994 (40 CFR Part 180.1150).

6-Benzyladenine and the end-use products Promalin and Accel were previously registered by Abbott Laboratories, Chemical and Agricultural Division, North Chicago, Illinois. This

division was purchased by Sumitomo Chemical Company of Japan in the year 2000, creating Valent Biosciences Corporation as a separate division of Valent USA, the US pesticide division of Sumitomo.

Promalin PGR: EPA Reg. No. 73049-41
Accel PGR: EPA Reg. No. 73049-29

These end-use products are registered in more than 25 countries around the globe.

In Appendix B copies have been provided of

- EPA Reregistration Eligibility Decision (RED) on N6-benzyladenine.
- California Department of Pesticide Registration notice of final decision on November 9, 1998 to register the end-use product Accel containing N6-benzyladenine.
- Federal Register, July 5, 1995 (Volume 60, Number 128) announcement of the tolerance exemption for 6-benzyladenine.
- A recent print out of the EPA Office of Biopesticide Programs Biopesticide Fact Sheet on Cytokinin, Kinetin and N6-benzyladenine.
- Print out of global registrations and EUPs for Valent Bioscience products containing 6-benzyladenine (6-BA).

8. The chemical Abstract Service (CAS) number.

6-Benzyladenine: CAS # 1214-39-7

9. The substance's physical properties and chemical mode-of-action, including:

A copy of the SRC PhysProp Database is included in Appendix C together with the MSDS.

6-Benzyladenine: molecular weight 225.25, melting point 233 degrees C, water solubility 60 mg/L, Log P (octanol-water) 1.57.

(a) Chemical interactions with other substances, especially substances used in organic production;

With the exception of Bacillus thuringiensis products DiPel, Xentari and Biobit for caterpillar control no other compatibility data have been generated. Bacillus thuringiensis products are suited for tank mixing since no toxicity to bees can be expected.

(b) Toxicity and environmental persistence;

As mentioned in the EPA Reregistration Eligibility Decision, N6-benzyladenine is a biochemical pesticide and therefore environmental fate studies were not required. Soil metabolism studies indicate that N6-benzyladenine has a half-life of 7 to 9 weeks.

With only two recommended applications at a the very low rates at which in general plant growth regulators are active, no accumulation of N6-benzyladenine can be expected during the growing season.
Since fruit thinning and sizing products can be applied during bloom, it is especially important to note that N6-benzyladenine is non toxic to foraging bees. N6-benzyladenine, with only slight toxicity to fish and aquatic invertebrates in laboratory tests, does not cause adverse effects to avian or aquatic species, when used at recommended rates.

(c) Environmental impacts from its use or manufacture;

The manufacture of N6-benzyl adenine is a very simple synthetic process with complete accountability of non-reacted materials by extraction and solvent recovery. The manufacturing document shows the steps involved and the possible formation of impurities and their estimated levels in the final technical grade material. No impact of N6-benzyladenine manufacture or from the use of this naturally occurring plant growth regulator on the environment can be expected.

The options for fruit thinning and crop management by non-organic growers are to use either carbaryl (Sevin insecticide with fruit thinning properties), naphthalene acetic acid (NAA), and N6-benzyladenine. Organic fruit growers do not have these options and use costly manual labor in crop management. The advantage of N6-benzyladenine is that there are no adverse effects on fruit size as has been observed with the use of naphthalene acetic acid (NAA). For organic growers the use of products with N6-benzyladenine as active ingredient would be a welcome fit in their limited arsenal of crop production aids.

(d) Effects on human health;

N6-benzyladenine has relatively low acute toxicity; the 50% oral lethal dose for rodent is 1.3 gram per kg body weight, with slight maternal toxicity shown in laboratory animals only. The eye irritation and dermal irritation tests showed moderate to slight irritation respectively. Dermal toxicity (rabbit) LD50 is > 5 grams per kg body weight and rat inhalation LC50 toxicity is 5.2 mg/Liter air. Three different tests for mutagenicity showed that N6-benzyladenine is not a mutagen. With only two recommended applications at a the very low rates at which in general plant growth regulators are active, no accumulation of N6-benzyladenine can be expected on fruit at harvest.

The en-use product Promalin, containing N6-benzyladenine as one of the active ingredients, has received from EPA the minimum labeling requirements for safety under the Worker Protection Standards (WPS) for agricultural uses.

(e) Effects on soil organisms, crops, or livestock.

As mentioned in the EPA Reregistration Eligibility Decision, N6-benzyladenine is a biochemical pesticide and therefore environmental fate studies were not required. Soil metabolism studies indicate that N6-benzyladenine has a half-life of 7 to 9 weeks. No effects on soil organisms have been documented. N6-benzyladenine does not cause phytotoxicity to crops and has no adverse effects on livestock.

10. Safety information about the substance.

Material Safety Data Sheets from two suppliers of the 6-benzyladenine active ingredient have been included in Appendix C. The substance was not listed with the National Institute for Environmental Studies.

11. Research information about the substance that includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies that present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List.

Attached are literature searches from Agricola, a combined Biosis-CABA-Agricola-HCAPlus search and 4 copies of references on the occurrence of benzyladenine by van Staden& Crouch (1996), Pechova, poster abstract (2001), Strnad, et al. (1992), Strnad (1996).

12. A "Petition Justification Statement" which provides justification for the inclusion of 6-benzyladenine on the National List.

Fruit thinning as indispensable tool for growers.

Thinning of apples is an important cultural practice for maximization of fruit size and quality. Also, adjusting of annual crop loads by thinning greatly aids in the prevention of alternate bearing (alternating seasons of heavy cropping, followed by little or no crop the following year).

Recently, the cytokinin plant growth regulator N6-Benzyladenine (6BA) was introduced as an alternative apple thinning product by Abbott Labs (now Valent BioSciences Corporation). Because 6BA has a non-toxic mode of action, impact on beneficial arthropods is minimal. Also, 6BA can effectively thin many varieties of apple with less risk of overthinning compared to NAA. 6BA increases cell division in the developing apple fruitlet during the critical growth period corresponding to 4-5 weeks following bloom. Thus, in addition to the apple sizing effect that can be attributed to its ability to reduce cropload, 6BA also has the advantage of directly increasing apple size through its cytokinin effect.

Novel and environmental safe crop management should be available to organic growers.

In non-organic apple production, carbaryl and NAA (napthaleneacetic acid) have been the most commonly used thinners. However, both products have limitations. Carbaryl, a synthetic carbamate insecticide, is toxic to beneficial arthropod species important to orchard Integrated Pest Management. NAA, a compound belonging to the auxin group of plant growth regulators, can cause overthinning, and can lead to reduced fruit size even when the appropriate thinning response is achieved, particularly in varieties prone to the formation of pygmy fruit.

Based on the advantages outlined for 6BA, this natural occurring active ingredient will become a very significant part of cultural management of apple production. Its addition to the National List will enable organic growers to benefit from the availability of products with 6BA such as Promalin (and Accel) and lower fruit production costs making those growers more competitive.

6-Benzyladenine

Appendix A

End-use product labels and technical bulletins

Promalin®

Technical Use Guidelines For Apples

Guidelines for Product Use

Promalin is a plant growth regulator used on apples to improve fruit shape (typiness) through elongation of fruit and development of more prominent calyx lobes. These desirable effects will be most evident in years when natural typiness is limited. Promalin may increase weight of individual fruit and yield per tree. Some thinning may occur from the use of Promalin.

Timing of Application

Split applications are used to improve typiness in locations where the bloom period occurs over a period of several days. Make two Promalin applications three or more days apart. The first spray should be timed during the first flush of bloom. A second application should be made when the remainder of the tree canopy comes into bloom.

The time between Promalin applications may vary from orchard to orchard within a given growing area because of the influence of the local microclimate. In most instances, a period from 3 to 7 days between sprays is adequate. The time interval between sprays should be lengthened if cool weather further prolongs bloom. For the most efficient use of Promalin, bloom progression within individual orchards should be assessed daily.

Application Rate

Pacific Northwest: 1.5 - 2.0 pints/acre in a single application

Rest of U.S.: 1.0 - 1.5 pints/acre total in either a single application or split applications

Note: Rates higher than 1.5 pints/acre in a single spray may cause some thinning in some areas outside the PNW.

Spray Volume

Apply Promalin in a sufficient amount of water to ensure thorough, but not excessive, coverage. Adjust water volumes based on tree size and spacing. A suggested spray volume of 75-150 gallons/acre is adequate for most orchards. Excessive spray application volumes that result in spray runoff may reduce product performance.

Application Considerations

1. For optimum effectiveness, thorough spray coverage must be achieved. All parts of the plant must receive the spray or desired results will not occur. On bearing trees, approximately 85% of the spray volume should be directed into the upper two-thirds of the trees.
2. Promalin applications made under slow drying conditions (cool to warm temperatures, medium to high relative humidity, and no wind) will increase absorption by the plant, thus optimizing effectiveness. Night-time applications are encouraged when day-time conditions are not conducive to slow drying conditions.
3. Rainfall within six hours after spraying may reduce the activity of Promalin.
4. Do not apply Promalin when air temperatures are lower than 40°F or greater than 90°F.
5. Do not apply Promalin in more than 150 gallons of spray per acre.
6. **Do not spray low vigor trees or trees under stress.**

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Apple Thinning Guide

Philip G. Schwallier

MSUE District Horticulture and Marketing Agent
Clarksville Horticultural Experiment Station Coordinator

April 1996

Preface

Apple Thinning Guide

I would like to dedicate this publication to my family

my wife, Judy,
and my children, Jody, Katie, Becky, & Timothy

for the support they have given me
over the years I have been an extension agent.

This publication would not be possible without many people that helped review this work. None of them reviewed the final draft; and thus, any errors or weaknesses are entirely my own.

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Belding, Grower

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My thanks to Rhone-Poulenc Company and Abbott Laboratories for their support of this publication.

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Apple Thinning Guide

Introduction

Many apple varieties have the ability to set heavy crops most years. This precocious trait to set heavy crops is not totally desirable due to the negative effects a heavy fruit set has on annual production and fruit quality. Chemical thinning is an important management practice used to control the tendency to overset heavy crops.

Thinning fruit has beneficial effects on apple production and fruit quality. Chemical thinning stabilizes annual crop production and improves size, color and quality of fruit. The trees response to chemical thinning, unfortunately, will often vary without any apparent reason.

Chemical thinning fruits early is desirable to improve size. Fruit size is directly related to the earliness fruits are thinned. Thinning that reduces clustering of fruit will improve fruit color and quality. Adequate chemical thinning will promote or guarantee return bloom, and promote consistent annual production of full crops. Thus, biennial bearing characteristics of certain varieties will be reduced by chemically thinning the fruit, especially when accomplished early.

Recently some new materials and formulations have become available to use as chemical thinners. This publication will help understand the factors involved in fruit set and thinning and help plan thinning decisions. It will review the conditions that impact the thinning response. A review of variety sensitivity helps guide rates and materials. The information included in this bulletin is only a guide of standard recommendations. As more experience is gained using these new thinners, adjustments will be made to these recommendations.

Past experience is the best guide for planning and applying thinners. Growers who have carefully studied and used thinners for several years will be the most successful in their thinning programs. Practice and keen observation with a basic knowledge of the factors involved with thinning will greatly improve the thinning success.

Because of the many variables, no single thinning program is applicable to all orchards. Over thinning or under thinning may result from the same application made on different orchards or may result from the same application made in different years. Records of past seasons' thinning practices, crop performances and crop management practices will help thinning decisions and successful thinning year after year.

First, an explanation of the thinning windows will help clarify important timing of thinner applications. A complete review of the factors that should be considered will help fine tune thinning rates. Weather factors are the most important factors to consider. The time and the kind of weather that occurs will greatly affect fruit set and therefore, thinning. The Fruit Set & Thinning Planner (Fig. 3), combines all these factors into a simple chart to organize all the complex factors.

Understanding what thinning materials are available and how they work will help make decisions on which ones to use and when to use them. Knowing the responses that can be expected from different varieties will greatly increase the success of thinning.

A final check of conditions at the time of thinning will improve thinning success. Proper evaluation of fruit set increases the success of the thinner or of the need to re-thin in some situations.

Thinning Windows

Chemical thinning can be accomplished in a short period from bloom to approximately 30 DAFB (days after full bloom). Some years this time of receptivity of the fruitlets to thinning is longer and some years shorter.

The receptive thinning period is divided into five sub windows. This is to make it easier to describe, communicate and understand the risks and the responses that can occur with thinning. These five sub windows are visually represented in Fig. 1.

Each sub window is approximately five days wide starting at petal fall. In any particular year, these sub windows can be shorter or longer depending on the weather that occurs. For example, when temperatures are warm, the fruitlets will develop more rapidly and the window will be shorter.

In the past, thinning was usually done during the Early Fruit Set Window (8mm to 12mm). This traditional thinning window has been a good target window and will continue to be a useful thinning window.

Under good growing conditions, developing fruitlets gain strength and will set. Their sensitivity to the stress of a chemical thinner or other environmental stresses decreases over time. Perhaps by 30 DAFB all fruits are beyond the point of being responsive to chemical thinning.

In the past, traditional thinning sprays were applied late in the thinning season and usually at moderate and mild rates. Today's demand for larger fruit and annual production require aggressive thinning and applications earlier after bloom.

The five thinning windows include Bloom, Petal Fall, Early Fruit Set, Late Fruit Set and Closing. The best general thinning occurs when thinners are applied in the Petal Fall window. Sometimes the weather is not favorable for thinning in that window. When this occurs, sprays would be applied as soon as possible when favorable weather returns after petal fall. A description of each window will help understand why early thinning is the best and what risks are involved in early thinning.

Bloom Window

In the western United States growers routinely use chemical thinners during the bloom period to reduce fruit set. All of their bloom thinning materials are phytotoxic to the plant.

The materials used reduce fruit set by preventing pollen germination or by preventing fertilization. Eastern United States weather and fruit set is not consistent or dependable and such material should not be used.

In New York, NAA is recommended as a bloom thinner for Empire and other small fruited varieties. When fruit set predictions warrant a bloom application on these varieties, NAA at 10 ppm can be applied as a dilute spray during bloom to do some limited thinning. Additional thinning is usually required following a bloom thinning application.

NAA is not compatible with Promalin or with Accel on Red Delicious and should not be applied on the same tree in the same year.

Some growers hand thin blossom clusters down to single king fruit on high value varieties such as Galas. Hand blossom thinning is labor intensive and is not feasible at current apple prices.

Considerable rainfall during the pink, bloom and post bloom (thinning window) period, may increase the thinner response. Adequate to abundant moisture will promote the growth of tender foliage and will increase the response to thinners. The cuticles that develop under moist conditions tend to be thin, increasing potential thinner absorption.

Petal Fall Window

Thinning during the Petal Fall window is the first chance to do general thinning on an orchard-wide basis of all varieties. All thinners can be used during the Petal Fall window. Petal fall is defined as the day when 80% of the flowers have dropped their petals on bearing wood. It is important to note that the bloom on one-year-old wood is not considered bearing wood. However, the bloom on the tips of one-year-old wood of terminal bearing varieties is important to consider.

Early thinning in the petal fall window will have the greatest affect on increasing fruit size. Early thinning will also improve fruit quality and increase return bloom.

At petal fall the important factors that influence fruit set and thinning can be evaluated. All the factors are listed in Table 1. Thinning and Fruit Set Factors. The factors are listed in order of importance. The main factors to consider at the petal fall stage include the quality and quantity of bloom, bee activity, pollination level, and the weather during pink and bloom.

Unfortunately, fruit set can only be predicted at petal fall. No evaluation of young fruit growth and development is observable for a few more days. The fruit set is really unknown and therefore applying thinners at petal fall based on a predicted fruit set is somewhat of a risk.

Use the Fruit Set & Thinning Planner (Fig. 3) to predict potential fruit set. Then the level of thinning can be determined based on the predicted fruit set. The weather that occurs now can greatly impact fruit set. Environmental stress to the fruitlets can cause the fruitlets to not set. The petal fall window is the best timing to do good thinning and have maximum effect on fruit production and quality.

Early Fruit Set

Early fruit set has been a traditional time to apply thinners. Typically this stage of development is between 5 and 10 DAFB and fruit size is between 8 and 12 mm. For years growers have successfully applied thinners during the early fruit set window.

Now, for first time fruit set can be observed. The fruitlets that are growing have the potential to set. In some years, fruitlets that are not setting can be readily visible. But in some years, fruitlets can be deceiving and not set without much visible indication.

Figure 1. Thinning Windows

This chart represents normal fruitlet development during the normal thinning period for the Peach Ridge Weather Station. The unique sub thinning windows are defined to better understand the timing of thinning applications. They are unique periods during fruit set and thinning. Early thinning will result in the best thinning success and overall annual fruit production.

Thinning Window (Sub Windows)	Bloom	Petal Fall	Early Fruit Set	Late Fruit Set	Closing
DAFB (Days after Full Bloom)		5 to 10	11 to 15	16 to 20	20+
Fruit Size (mm)		PF to 8	8 to 12	15 to 20	20+
Stage (mm)	Full Bloom, Petal Fall	6	8, 10	15, 20	25
Date	18, 19, 20, 21, 22, 23	24, 25, 26, 27, 28	29, 30, 31, 1, 2	3, 4, 5, 6, 7	8, 9, 10, 11, 12, 13, 14, 15
(Month)	May	May	May / June	June	June
General Thinning Success	Fair	Best	Good	Good	Ok
Past Thinning			Traditional Window		Rescue Thinning

Weather conditions during early fruit set continue to have great influence on developing fruitlets. Poor growing conditions can cause fruitlets to drop but growing fruitlets will continue to gain strength with time and be more tolerant of environmental stress that may occur.

If thinning is needed and no thinning sprays have yet been applied, serious consideration should be given to making an application. The fruitlets are now at an early stage in their development and will respond to thinners better at this early stage than applications made later (Fig. 1).

Late Fruit Set

During the late fruit set window fruitlets are from 12 to 15 mm in size. Often it is 11 to 15 DAFB. During the late fruit set window is the last chance to accomplish good thinning and good crop response. Thinning can still be accomplished in the closing window, but good results are not always achieved.

The fruitlets are gaining strength and will be more difficult to thin than earlier in their development. The leaves are older, less tender, waxy and will resist absorption of thinners.

Although the weather during pink, bloom and petal fall, has significant effect on the developing fruitlets, the growing fruitlets are becoming more independent of those past conditions and gaining their own strength. The most important factor to consider at this time is the observable fruit set with less emphasis on past weather conditions.

Closing

During this time of the thinning period, the receptivity and response to thinners is declining. Often thinning during this stage is considered rescue thinning. Fruitlets are strong and leaves are older, waxy, and resistant to absorption of thinners.

Thinner applications that use combinations are the only sprays that will thin aggressively. Moderate, mild or no thinning is usually all that occurs when thinners are applied late in the thinning season.

Thinning Factors

There are many factors that influence the absorption, sensitivity and response of chemical thinning. Often similar thinning applications and conditions cause different responses. Because of the somewhat nonrepeatability of thinning, an understanding of these factors still does not guarantee perfect thinning, but thinning will be improved. The factors that influence fruit set and thinning response are listed in Table 1. A review of these factors will help understand thinning and help plan applications. **The one factor that is important above all other factors is the temperature at the time of applying the thinning spray.**

Bloom Factors

Proper evaluation of bloom can enhance the success of thinning. Large showy bloom is usually an indication of strong healthy flowers and potentially a heavy set. When abundant amounts of foliage are present during the bloom stage the fruit set will be stronger.

The foliage present with bloom greatly strengthens bloom. Undamaged foliage that is healthy and develops early in the growing season is the major supplier of energy to bloom and growing fruitlets.

Abundant amounts of bloom can be misleading. An abundant bloom is often described as a "snowball" bloom, but a "snowball" bloom can be at opposite extremes of strength for setting fruit. Some "snowball" bloom is strong healthy bloom and sometimes "snowball" bloom is weak and small.

When the bloom is "snowball" and weak, the trees are white with bloom and have very little green foliage showing. The trees are gorgeous to view, but much of the tree's energy is put into flower growth. The bloom is weak, smaller in size, has missing or incomplete parts, is very competitive with other flowers and doesn't set fruit well. Fruitlets in this situation are easily thinned. Reduce the strength of the thinner application when a weak "snowball" bloom occurs.

A heavy bloom after a heavy crop is usually weak bloom and will not set abundant amounts of fruit. The fruit is easy to thin.

When the bloom period lasts a short time, pollination and/or fertilization is usually inadequate. A quick bloom occurs with hot temperatures and the fruit set is poor and fruitlets will thin easily.

When thinners are applied during bloom, such as NAA on Empire, fruitlets are often more difficult to thin with a second application of thinner applied late in the thinning period. Blossoms injured by frost will thin easily.

Bee & Pollination Factors

Having adequate bee numbers is more critical today than even just five years ago. Wild bee populations used to supply 1/2 to 2/3 of the pollinating bees. Due to mite infestations, the wild colonies are near zero. Improve fruit set and fruit quality by placing an adequate number of bee colonies in the orchard to accomplish excellent pollination.

Abundant, active bees will pollinate a fruit crop in a short time. Multiple bee visits per flower are necessary to pollinate and then fertilize flowers. The greater the bee activity, the greater the number of fruits set and potential for a heavy crop.

Windy conditions during bloom will reduce bee activity and pollination. Also, windy conditions will dry out the stigmas on flowers, pollen will not germinate well and grow normally, reducing potential set. When poor bee activity or conditions occur during bloom, fruit set can be quite poor. This becomes a major factor to consider.

Honey bees will not work bloom well during hot conditions because they are busy collecting water to cool their hives. Providing water close to hives and shade for hives may improve bee numbers working bloom.

Competitive bloom, such as dandelions, will often attract bees if apple flowers are not attractive enough. This competitive bloom will draw bees out of the fruit trees but it must be extremely competitive to impact pollination or bee activity in the trees negatively.

Bee activity only needs to be adequate to set a crop. Good to excellent fruit set can be accomplished in a short time of just a few hours of adequate bee activity.

Pollen quality is not easily judged but in general is not a concern most years. Some years pollen can be easily rubbed off stamens with the tip of your finger. That is an indication of abundant healthy pollen.

Pink & Bloom Weather Conditions

The weather conditions during the pink and bloom period can be an excellent guide to predict fruit set and thinner response. Good conditions will strengthen fruit set and poor marginal weather will reduce fruit set.

Excessive cool wet cloudy weather during this stage will reduce fruit set

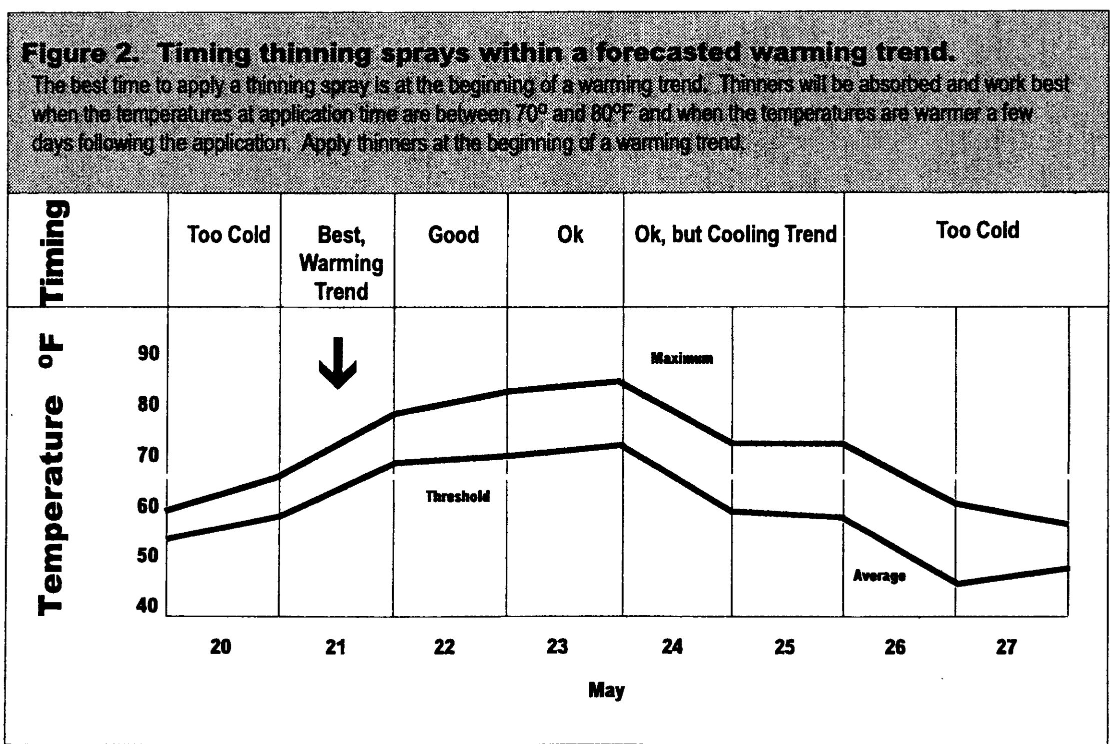


Figure 2. Timing thinning sprays within a forecasted warming trend. The best time to apply a thinning spray is at the beginning of a warming trend. Thinners will be absorbed and work best when the temperatures at application time are between 70° and 80°F and when the temperatures are warmer a few days following the application. Apply thinners at the beginning of a warming trend.

Figure 3. Fruit Set & Thinning Planner

These are the important factors that influence fruit set and response to thinners. Complete this chart during the late bloom stage as a first step to determine the thinning level needed. For example, if most of the checks are in the aggressive thinning level class, the predicted set is high and the thinning strategy would be to use aggressive rates and timing. If some of your checks fall below this class, then consider using a reduced thinning level. Be ready to apply the predicted thinning level needed at petal fall if weather is favorable.

✓ Check off factors that best describe the situation.

Thinning level.	Predicted Set.	Bloom Factors.	Pink & Bloom Weather Conditions.	Bee & Pollination Factors.	Grower Management Factors.	Tree & Orchard Factors.
Aggressive	**Good to heavy fruit set.**	No king flowers missing. Healthy flowers, especially king flowers. Abundant large showy blossoms. Healthy petals, pistils, stamens and anthers. Abundant and healthy foliage present with bloom. ☐	Mostly ideal temperatures between 60° and 80°F. Mostly sunny weather. Adequate moisture levels. No frost. ☐	Excellent bee activity. Excellent calm conditions. Excellent pollen and nectar quality. ☐	Past experience, difficult to thin block. Difficult to thin variety. Small fruited variety. Strong biennial tendency. Light crop last year. ☐	Fruitful trees and at mature bearing habit. Well pruned and no shaded fruit buds. No winter damage. No disease damage. Excellent nutrient levels especially nitrogen and boron. Correct soil pH levels. ☐
Moderate	**Moderate to good fruit set.**	Occasional king flower missing or damaged. Normal to large sized bloom. Most flower parts healthy, except for occasionally deformed or missing flower part. No frosted flowers. ☐	Mostly favorable conditions. Some cold or hot temperatures. Some cloudy or rainy weather. Very dry or wet. Some windy conditions. ☐	Mostly good bee activity. Some windy conditions. ☐	Mature fruiting wood and/or fruitful trees (normal vigor). Normal or moderate crop last year. ☐	Lateral growth habit. Minor winter damage. Minor disease damage. Old age fruit wood. Normal nutrient levels. ☐
Mild	**Fair to moderate fruit set.**	Some king flowers missing. Short or quick bloom period. Inconsistent bloom. Weak "snowball" bloom and/or very light bloom. Small amounts of foliage present with bloom. Some frost damaged flowers. Incomplete flowers. ☐	Unfavorable conditions. Some light frost. Mostly rainy and cloudy. Mostly cold or hot temperatures. Excessive soil moisture or excessively droughty. Excessive windy conditions ☐	Fair bee activity. Some excessive windy conditions. Pollen quality is questionable. Abundant competitive bloom sources. ☐	Heavy crop last year. Past experience, easy to thin. Low vigor orchard. ☐	Fruiting wood young (young trees). Low vigor or upright growth habit. Some winter or disease damage. Some leaf damage and/or small leaves. Some low nitrogen or nutrient levels. Acid soil pH. ☐

and make thinning easier. Cool wet and cloudy weather will promote the development of succulent tender foliage that readily absorbs thinners. The cool cloudy weather promotes the absorption of thinners because the leaf cuticle is thin and less waxy. Also, fruitlets are stressed from the cloudy weather and may not set well.

Hot, sunny and dry conditions will make thinning more difficult. The cuticle will develop a thick waxy layer resistant to penetration by thinners, reducing a thinner's response.

Extreme conditions of heat, cold, wet or dry will decrease fruit set. The stress of the extreme conditions weaken the fruitlets and they do not set and/or are easily thinned. In these situations reduce thinner strength.

Grower Management Factors

These factors are quite important for successful thinning. Past thinning results provide the best bench mark to judge a block's sensitivity to thinners.

Blocks that have been difficult to thin and/or have strong biennial bearing tendencies should automatically be in an aggressive thinning level. Biennial varieties should be thinned aggressively, especially the year after a light crop. Blocks easy to thin should be carefully evaluated before an aggressive approach to thinning is used.

When fruit set is heavy on easy to thin varieties, generally the fruitlets will be easy to thin. The competition between growing fruitlets will weaken all the fruitlets and each fruitlet will be quite sensitive to thinning stresses and for that matter, environmental stress as well.

Large fruited varieties still need thinning to produce quality fruit and annual crops. Clustered fruit of any size does not mature correctly and colors poorly. Small fruited varieties need to be thinned early to gain the best potential for larger fruit size.

Thinning after a light crop can be difficult. The trees are rested and have stored more energy in wood and buds for this year's crop. These trees will generally be more difficult to thin.

Using high concentrations of thinners in small amounts of water will increase the thinning response. Concentrations higher than 3X are unpredictable and non repeatable and should be avoided. Use more dilute applications to get a predictable response. Hard spray water will decrease the response of the thinner.

Orchard Factors

Many of the factors listed under grower management factors and under orchard factors could be listed under either category. But to help clarify certain characteristics, and because trees and blocks change over time, they have been separated into two categories somewhat arbitrarily.

Trees that are fully mature and in full bearing are more difficult to thin. These types of trees will tend to set considerable numbers of fruit and need aggressive thinning. An immature fruiting habit of young trees and/or young upright growth does not set well and is easy to thin. Young trees bearing their first and/or second crop usually fall in that category. Moderate, more lateral growth is difficult to thin. The excessive upright growth and low vigor growth thins easily.

Low nutrient levels of N (nitrogen) and B (boron) are especially important to fruit set. Fruits weakened by low N and/or B, will thin easily. Low soil pH levels usually are easy to thin due to the potential nutrient imbalance in the tree and/or the stress a tree may be under.

Trees that have had excellent pruning and training to provide an open tree form with good exposure to sunlight will tend to set abundant fruit and need more aggressive thinning. Shaded areas in trees or between trees thin easily. This is usually the case with the lower half of semidwarf and seedling trees. In many years no thinners should not be applied to the lower half of these larger trees.

Diseased or injured trees thin easily. The weakened wood will have less energy reserves and the flowers will be more competitive with each other. The fruitlets will respond to thinners and other stresses that occur at fruit set time. If the early developing foliage is injured by weather or chemicals, fruit set is reduced and trees are easy to thin

Weather Factors

The weather that occurs at various times during the growth and development of foliage, bloom and fruitlets is the main driving factor to consider when preparing a thinning strategy and carrying it out. The most important weather factor to consider when applying thinners is the temperature. Also the drying time of a spray application is important to consider. Warmer temperatures and slow drying times increase thinner absorption.

Temperatures

The temperature during a thinner application and the temperature after the application are critical to successful thinning. In order for thinners to work they must be absorbed. Absorption increases as the temperature increases. All thinners have greater response as application temperatures increase. **The one factor that is important above all other factors is the temperature at the time of applying the thinning spray and the temperatures following the application.**

Temperatures below 65°F are considered to be too cold to apply thinners. Under cold temperatures absorption is significantly reduced and poor or no thinning occurs. All thinners work well when temperatures are in the optimum range of 70° to 80°F. When temperatures rise above 85°F, however, thinning can be greatly increased.

Extremely hot temperatures will stress growing fruitlets and set will decrease. If temperatures are forecasted to rise above 85°F the few days following a thinner application, then a greater thinning response will occur. Reduce dosage rates if increased thinning is not desired and/or delay the application. Approach hot temperatures with caution and consider discontinuing or delaying thinner applications, or decreasing the thinner strength.

Figure 4. Apple Thinning Rates and Timing.

Timing	Thinning Level Needed: Aggressive — Materials	Rates	Moderate — Materials	Rates	Mild — Materials	Rates
Petal Fall	1. Sevin + NAA	Full + 1/2 rate.	1. Sevin	Full rate.	1. Accel	50 ppm.
	2. Sevin + Accel	Full + 50 ppm.	2. NAA	2/3 rate.		
	3. NAA	3/4 rate.	3. NAD	Full rate.		

Apply one of the thinner sprays listed above in the Petal Fall window during favorable weather.

Application				↓																		
Stage (mm)	Petal Fall			6			8			10				15				20			25	
Window	Petal Fall						Early Fruit Set					Late Fruit Set					Closing					

Timing	Aggressive — Materials	Rates	Moderate — Materials	Rates	Mild — Materials	Rates
Early Fruit Set	1. Sevin + NAA	Full + 2/3 rate.	1. Sevin + NAA	Full + 1/2 rate.	1. Sevin	Full rate.
	2. Sevin + Accel	Full + 50 ppm.	2. Sevin + Accel	Full + 50 ppm.	2. Accel	50 ppm.
	3. NAA	Full rate.	3. NAA	3/4 rate.	3. NAA	2/3 rate.

Apply one of the thinner sprays listed above in the Early Fruit Set window during favorable weather.

Application									↓													
Stage (mm)	Petal Fall			6			8			10				15				20			25	
Window	Petal Fall						Early Fruit Set					Late Fruit Set					Closing					

Timing	Aggressive — Materials	Rates	Moderate — Materials	Rates	Mild — Materials	Rates
Late Fruit Set	1. Sevin + NAA	Full + 3/4 rate.	1. Sevin + NAA	Full + 2/3 rate.	1. Sevin	Full rate.
	2. Sevin + Accel	Full + 75 ppm.	2. Sevin + Accel	Full + 50 ppm.	2. Accel	50 ppm.
			3. NAA	Full rate.	3. NAA	3/4 rate.

Apply one of the thinner sprays listed above in the Late Fruit Set window during favorable weather.

Application														↓								
Stage (mm)	Petal Fall			6			8			10				15				20			25	
Window	Petal Fall						Early Fruit Set					Late Fruit Set					Closing					

This chart is a starting point for planning thinning rates and timing at various thinning levels. The stages and application windows are timings based on normal fruit development for the Peach Ridge Weather Station. Each square indicates a normal day in apple development. The shaded (yellow) area is an indication of the appropriate timing window and the down arrow suggests that a thinning spray should be applied in that window when weather is favorable. Basically, early applications of good rates thin aggressively. Moderate and mild thinning occurs at lower rates and/or at later timings. The rates of NAA used alone and/or in combinations should be based on past grower experience or use variety and rate guidelines (Table 2).

☐ **Timing Window**

↓ **Apply Spray**

Humidity

To thin fruitlets chemically, absorption of the thinner is required. The absorption of the thinner mostly occurs during the drying time of the spray droplets. Almost no absorption occurs after the droplets have dried.

Slow drying conditions (such as high humidities), increase absorption of thinners and fast drying conditions (such as low humidities), decrease absorption of thinners. The humidity at the time of the thinner application can impact the amount of thinner absorbed and therefore the thinning response.

High humidities and dilute applications will slow the drying time and increase absorption, increasing the amount of thinning.

Applying thinners late in the day or at night may result in chemical thinners remaining wet all night on the tree. These potential long drying times will increase absorption and thinning. Adjust rates accordingly with the potential drying time.

Precipitation

Like high humidities, precipitation during and/or shortly after a thinner application can increase the drying time or re-wet dried thinners on the leaf. Increased thinning can result from such an event. Under cloudy conditions, thinner applications usually dry at a slow rate, increasing absorption and thinning.

Generally if thinners have two hours to dry before a rain there is no additional absorption of the thinner. Rain received within the two hours of the application can reactivate the thinner and increase the thinning response.

Cloudy Weather

Fruit set is influenced by stress that occurs during the fruit set period. Mostly or excessively cloudy conditions during pink, bloom and post bloom (thinning window) will reduce fruit set. Also, under cloudy conditions, leaf cuticles are thin and absorption of thinners increases.

Under such conditions fruitlets are easy to thin. Natural fruit set is reduced, fruitlets are somewhat weaker, and the leaf cuticle is thinner. Excessive thinning can easily occur. Cloudy conditions by themselves can over-thin the crop.

Sunny Weather

Fruit growth and development is driven by energy derived for the sunlight captured by leaves. The greater the energy captured the greater the strength and development of the fruitlets. Just as cloudy conditions decrease fruit set, sunny conditions promote fruit set. Sunny and hot conditions also increase the waxy cuticle of foliage and thus reduce absorption of thinners. Under such conditions strengthen thinners to compensate for the decreased sensitivity.

Materials

The materials available for thinning are limited to four. Some of these materials are dose dependent. The dose dependent materials work best when adjusted and applied according to the grower experience in the past.

Probably the rates growers use, based on their past experience, reflects their spray coverage and their application techniques more so than other factors of rate. The university rates are developed under full dilute applications and serve as benchmark rates to use until individual block rates can be established from years of experience.

Sevin (carbaryl)

Sevin was introduced as an insecticide in 1958. Shortly after its introduction, it was discovered to have thinning properties. It then became a regular thinner and with somewhat reliable characteristics. Today Sevin is classified as a mild reliable thinner.

There are three formulations available today, 50 WP, 80 SP, and XLR Plus. All the formulations thin with the same degree of effectiveness. Currently, Sevin XLR Plus is the preferred formulation for thinning. It is ground to a very fine texture to improve it performance.

Sevin has a limited dose response at low rates. It seems to behave as a dose dependant material up to point where the solution is saturated. Any additional concentration in the solution is held in suspension. From that point the increased concentration has no dose dependent effect.

Increasing the Sevin rate does not result in increased thinning. The dose response at the low rate has been proven to be of no importance and should not be considered when choosing a Sevin thinning rate. Sevin is a mild thinner at full rate and a reduced rate of below 1/2 rate is considered noneffective.

The recommended rate of Sevin XLR Plus is one quart per acre regardless of variety, tree size or tree density. Apply Sevin in 30 to 100 gallons of water per acre. A dilute application is preferred. Use the lower gallonage on smaller trees.

Sevin can be used starting at petal fall up to approximately 21 days after bloom. **All honey bees, however, should be removed from the orchard to avoid bee kill before any Sevin is applied as a thinner.**

Like other thinners, Sevin works best when applied under warm temperatures and early in a warming trend (Fig. 1). Temperatures above 85°F during and after the application will favor the thinning response.

Sevin works as a thinner in a very wide window on apples. It can thin apples from bloom to perhaps 30 DAFB (days after full bloom). Fruit that are larger then 25 mm generally are not responsive to an application of Sevin.

Sevin is a mild thinner that is gentle to fruit and foliage. It does not injure the plant. Research indicates that it is primarily absorbed through the fruit rather than the leaves. Sevin in the vascular tissues stress individual fruitlets by reducing the growth processes of those fruitlets and the fruitlets usually abscise in five to 10 days. Rarely does Sevin overthin but sometimes it does not thin enough. The thinning response is not easy to predict.

Sevin works best when applied under warm slow drying conditions.

Table 1. Thinning and Fruit Set Factors (in order of importance).

The following factors influence fruit set and the effects of chemical thinning and should be considered when making thinning decisions. For example, when a weak "snowball" bloom occurs, generally fruitlets thin easier and/or fruit set is lighter.

Increased thinning response.	Decreased thinning response.
Bloom	
Heavy or "snowball" bloom.	Light bloom.
Quick or short bloom.	Normal bloom period (good cross pollination).
Injured bloom or missing flower parts.	Healthy or large showy bloom.
Little or no foliage present during bloom.	Abundant foliage present during bloom ("green bloom").
Bees & Pollination	
Poor bee activity	Good bee activity.
Poor pollination and fertilization.	Good pollination and fertilization.
Pink & Bloom Weather	
Cool, wet or cloudy weather during.	Warm, mostly dry or mostly sunny weather.
Excessive hot temperatures.	Warm temperatures
Cold or frosty temperatures.	No frost.
Excessive moisture.	Mostly dry, but also adequate moisture.
Grower Management Factors	
Previous heavy crop.	Previous light crop.
Heavy fruit set on easily thinned varieties.	Light fruit set.
Low levels of N and/or other nutrients.	Adequate levels of N and other nutrients.
Wetting agent.	No wetting agent.
High chemical concentration.	Lower chemical concentration.
Soft spray water.	Hard spray water.
Easy to thin varieties.	Hard to thin varieties.
Tree Factors	
Excessive shading and/or crowded trees.	Well pruned and/or trained trees (open trees).
Close tree spacing.	Wide tree spacing.
Injured or diseased tree parts.	No disease or injured tree parts.
Young trees.	Mature bearing trees.
Winter injury.	No injury.
Mostly upright growth.	Mostly lateral growth.
Low vigor.	Moderate vigor.
Light pruning	Heavy pruning.
Non-spur type trees.	Spur type trees.

The weather during and just after the thinning application is the most important factor to consider in predicting the thinning response.

Increased thinning response.	Decreased thinning response.
Weather during and after thinner application.	
Slow drying conditions.	Fast drying conditions.
High humidities.	Low humidities.
Frosty nights.	No frost.
High maximum temperatures.	Lower maximum temperatures.
Mostly warm to hot temperatures (70° to 80°F).	Mostly cool temperatures (<70°F).

Figure 5. Fruit Set Evaluation Checkoff

Unfortunately fruit set is difficult to evaluate. This list of fruit set descriptions provides a general guide to the level of thinning needed to set a desirable crop. After completing this chart, use Figure 6, Final Pre-spray Checkoff, to apply the thinner spray.

Check off factors that best describe the situation.

Potential	Fruit Set Evaluation.	Management Thinning Action.
High Fruit Set	All or most fruitlets growing. ☐ Three or more fruitlets per cluster appear to be setting. Most clusters have growing fruitlets. Heavy density of growing fruitlets. Most kings setting. Leaves are healthy and no damaged fruitlets. Fruitlets curving upward and green colored stems. Many sepals folding closed over the calyx.	☐ **Fruit set is too heavy. Consider an aggressive thinning application, especially if in the Late Fruit Set Window.**
Good Fruit Set	Some fruitlets growing. ☐ Some kings not setting. Moderate density of growing fruitlets. Moderate amount of lateral fruitlets where kings not setting. Many fruitlets growing.	☐ **Fruit set is good to heavy. Consider a moderate thinning application.**
Fair Fruit Set	Few kings setting. ☐ Less than two fruitlets per cluster appear to be setting. Many clusters have no growing fruitlets. Light density of growing fruitlets. Yellow or red colored stems and fruitlets Many sepals remaining spread open at calyx.	☐ **Fruit set is near right or good. Consider a mild thinning application.**

Try to time the application at the beginning of a warming trend and when temperatures are over 70°F. There is some data indicating that when Sevin is applied at temperatures below 65°F, it still provides some thinning in some trials. Sevin can be used on all varieties and should be used at full rate. Temperatures above 85°F will increase the thinning response of the fruitlets. Sevin can be re-wetted and have an additional thinning effect, especially when using Sevin XLR Plus at the one quart per acre rate.

Sevin combined with other thinners such as NAA or Accel will thin aggressively. When aggressive thinning is required apply the combinations, but adjust the NAA or Accel rates based on the degree of thinning needed or desired. Some growers have reported over thinning using combinations of Sevin + NAA or Sevin + Accel.

When thinning late in the thinning period such as in the Late Fruit Set Window or the Closing Window, the combinations of other materials with Sevin are the only thinning sprays that will thin significantly.

Accel (benzyladenine)

Abbott Laboratories introduced Accel as a new thinner in 1994. Accel is a mixture of 6BA (benzyladenine) and GA_{4+7} (gibberellins). Accel has some interesting effects on apple growth and development.

It thins over a wide window, probably bloom to 30 DAFB, and promotes cell division over the entire window as well. It may have the widest window of thinning of all the available thinners. It is a gentle, very mild thinner and is dose dependent.

Like all thinners, it works best when applied during warm temperatures and early in a warming trend.

It can be applied to all varieties but has an excellent track record on small fruited varieties and some inconsistent results on other varieties. Varieties that Accel works well on include Empire, McIntosh, Rome, Idared, Jonagold and Jonamac. Accel usage on other varieties such as Red Delicious, Golden Delicious, Gala, Fuji, Jonathan, Paulared, Northern Spy, Cortland, Braeburn, and Spartan need further testing.

Accel is dosage dependent and will give an increasing response by increasing the concentration or increasing the rate per acre. Apply Accel under slow drying conditions and warm temperatures (70° to 80°F). The standard rate is 50 ppm (20 g/acre), but when more than just very mild thinning is needed increasing the rate to 75 ppm (30 g/acre) will increase the thinning. Apply Accel in 100 gallons of water per acre to achieve thorough coverage and help achieve slow drying.

Combinations of Accel with Sevin will give an additive thinning response. This combination is considered a strong aggressive thinner. Some growers have reported over thinning using this combination.

Accel is not compatible with NAA or NAD on the same tree within the same year. On Red Delicious when Accel (or Promalin) is applied on the same tree with NAA or NAD, fruit can be stuck on the tree and pygmy fruit are produced.

NAA

Naphthaleneacetic acid (NAA) has been used as a thinner for a long time. It should be applied under fast drying conditions. It is a reliable thinner but does have some unique characteristics. Varieties differ considerably in their response to NAA sprays. Increasing the dosage and/or increasing the absorption of NAA will increase the thinning response on all varieties.

NAA is a harsh thinner. It thins fruits by stressing the fruitlets and leaf tissue. The stress causes weaker fruitlets to drop and stronger fruitlets to remain and develop.

NAA, like other thinners, work best when applied under warm weather conditions and in a warming trend. NAA can be phytotoxic to the foliage when very hot (85°F) temperatures occur at the time of the application or in the days following an application. Adjust rates of NAA based on the performance of NAA in

Table 2. Varieties and Thinning Rates

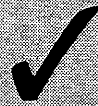
Varieties differ in their response to thinning materials. Use the following list of varieties to adjust your thinning rates for NAA. The following list is a starting point for using various materials. Experience thinning your own blocks will help fine tune the rates you need.

Easy to thin	Intermediate to thin	Difficult to thin
Braeburn Cortland Empire Gala Ginger Gold Idared Jonagold Jonathan Northern Spy McIntosh Red Delicious Winesap	R.I. Greening Jerseymac Jonamac Paulared Spartan Spur Red Delicious	Fuji Golden Delicious Lodi Rome Wealthy

Material	Rate: Concentration	Rate: Per Acre	
Sevin XLR Plus		1 qt.	
Accel	50 ppm	20 g	
NAD	50 ppm		
	Easy	Intermediate	Difficult
NAA	10 ppm	15 ppm	20 ppm

Figure 6. Final Pre-Spray Checkoff

Just before making any thinning application, the most important information to consider is the current and future weather. If good to excellent weather conditions exist and will continue for a few days, apply the thinner and good thinning response should occur. If weather is unfavorable for fruit set, delay the application or reduce the strength unless in a late thinning window.



Check off factors that best describe the situation.

Current & Five-Day Weather Forecast	Prediction	Management Thinning Action
☐ Mostly sunny weather. Mostly warm ideal temperatures, between 70° and 80°F. Normal or minor rain events. No extreme temperatures.	**Conditions are good to excellent for fruit set. Young fruitlets will be gaining strength.**	☐ **Apply the thinner and/or strengthen the thinning level.** **(If strengthening the thinning level and using NAA, strengthen the NAA rate by 25%.)**
☐ Mostly cloudy weather. Some extreme temperatures, especially cold temperatures. Hot temperatures of 85°F and above.	**Conditions are fair for fruit set. Set of young fruitlets is questionable.**	☐ **Apply the thinner and/or consider a reduced rate and/or delay the application.** **(If reducing the thinning level and using NAA, reduce the NAA rate by 25%.)**
☐ Mostly cloudy with considerable rain. High humidities. Mostly extreme temperatures. Frost and/or freeze possible.	**Conditions are poor for fruit set. Some fruitlets will not set.**	☐ **Delay decision until next thinning window of favorable weather.** **(If already late in the thinning season, delaying the thinning application may not be a choice.)**

your own blocks. Use the rates in Table 2 as benchmark rates or starting point rates in blocks where past NAA performance is unknown.

Some varieties are more difficult to thin than others. Use the higher rates on those more difficult varieties. When strengthening an application to cause greater thinning, increase NAA by 20 to 25% from the base rate. Increasing NAA rates by small increments (such as 1 ppm) is of little value and will not cause any detectable difference in response.

NAA can be used in combination with Sevin. The combination will result in additive thinning. The combination of Sevin + NAA is the strongest of all thinning sprays. Often this combination spray is the last hope of thinning when thinning in the Closing Window.

Accel and Promalin are not compatible with NAA or NAD on Red Delicious. Do not use on the same tree in the same year.

NAD

Naphthaleneacetamide (NAD) is a milder formulation of NAA. It also thins to a lessor amount than NAA. It should only be used in the petal fall window and it is a moderate thinner at that time. Do not use NAD later than petal fall or pygmy fruit may result.

It is recommended for use on varieties that mature before McIntosh. Use it at the standard rate of 50 ppm rate on all varieties. Apply NAD under slow drying conditions. Often an additional thinning is required later in the thinning period after an NAD petal fall spray.

Concentrate Thinning

All thinners should be applied as dilute as possible to achieve the best coverage and the best response. Dilute is defined as the amount of water per acre to thoroughly wet the tissue to the point of runoff. Mainly NAA is major thinner of concern when concentrate thinning.

Consider concentrating thinners only to the level of 2X or 3X. A 2X spray is using half the amount of water of a dilute application on a per acre basis. If 10 ppm of NAA were used in the dilute application, then a 2X application of the water would have 20 ppm of NAA if no change were made to the NAA rate. Reduce the NAA concentration in the water as the rate of water is reduced.

Concentrating thinners is dangerous and unpredictable. **Concentrations of 3X or higher are experimental.**

Surfactants

In order to thin fruitlets absorption of the chemical thinner is necessary. The addition of a surfactant will increase the absorption and the thinning response. Use of a surfactant is not normally recommended, but it can be useful under conditions that reduce absorption.

The possible poor absorption conditions may include windy and very low humidity conditions. Foliage with thick waxy cuticles reduce absorption. A surfactant would improve absorption. A surfactant might be of use on a difficult to thin variety to improve thinning.

In general, surfactants add another variable to the thinning process, a process that is already complicated enough without additional variables.

Fruit Set Evaluation

Evaluating fruit set is a difficult task in the early development of the fruitlets. On some varieties (spur type varieties), many spurs need to rest in order to achieve annual regular production. These varieties are prone to biennial bearing.

For Red Delicious, more than 1/2 of the spurs should not be allowed to set fruit. These resting spurs will then produce flowers for the following year. Other varieties with biennial tendencies need this same level of thinning.

Generally, an excellent set is evenly spaced out king fruits. The spacing between fruits will depend on the variety and the size fruit trying to be grown. Individual fruits should be 4" to 6" apart to gain good size of high quality.

Fruit that is clustered produces fruit of lower quality. Thin to reduce clustering to a minimum. Certain varieties are prone to the clustering phenomenon. Paulared, Golden Delicious, and Rome are examples of the clustering type. These varieties are all in the intermediate or difficult to thin class. Thin these varieties more aggressively.

At the early fruit set window is the first chance to evaluate fruit set. Fruitlets that are setting include:

1. Fruitlet stems that curve upward to the sun.
2. Fruitlets that are steadily increasing in diameter.
3. Fruitlets and stems that retain a green color.
4. The sepals fold in and close over the calyx end.

Fruitlets that are not setting include:

1. Fruitlet stems that remain straight.
2. Fruitlets that stop growing.
3. Fruitlets and stems that have yellow or red color.
4. The sepals remain folded open.

When fruit develop a separation of sizes on the tree, the larger fruit are setting. The medium size fruit may set and the small fruit will most likely not set. It is advisable to apply thinners to make sure the smaller fruit drop if fruitlets are too numerous. Oftentimes it is desirable to thin the medium sized fruitlets as well. Use a stronger thinning rate to achieve thinning of medium sized fruitlets.

When fruitlets develop curved stems upward toward the sun, they are setting. Straight stems may indicate a non-setting fruit. Yellow and red stems and fruitlets are signs that the fruitlets are not setting. When sepals do not close over the calyx, but remain spread open, usually the fruitlet is not setting.

Setting fruit will grow in diameter every day (approximately one mm per day). Fruitlets will grow slowly under cold weather conditions and daily size changes are less evident. Marking fruitlets and recording their growth over time will help predict fruit set. Fruitlets that are not setting will fall behind setting fruit.

Basically counting the number of fruitlets setting on a length of fruiting branch is the guide used to determine the level of thinning needed.

Summary

Aggressive and early thinning will generally provide the best success of annual fruit production and fruit quality. Review the factors that influence fruit set and thinning. Select materials, rates and timing to achieve the desired thinning level needed each year. The best information to follow is past experience. Try new rates and timings on a small scale to gain experience in thinning.

Promalin®

Plant Growth Regulator
Solution

For use on apples, non-bearing pears, and non-bearing sweet cherries.

ACTIVE INGREDIENTS:

N-(phenylmethyl)-1H-purine 6-amine	1.8% w/w
Gibberellins A_4A_7	1.8% w/w
OTHER INGREDIENTS	96.4% w/w
TOTAL	100.0% w/w

Store below 75°F (24°C)

EPA Reg. No. 73049-41
EPA Est. No. 33762-IA-001 List No. 2571

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KEEP OUT OF REACH OF CHILDREN

CAUTION

1.0 FIRST AID

If in eyes	• Hold eye open and rinse slowly and gently with water for 15-20 minutes. • Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. • Call a poison control center or doctor for treatment advice.

HOT LINE NUMBER

Have the product container or label with you when calling a poison control center or doctor, or going for treatment. For medical emergencies, you may also call toll-free 1-877-315-9819 for treatment information.

2.0 PRECAUTIONARY STATEMENTS

2.1 Hazard To Humans and Domestic Animals

CAUTION

Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling.

2.2 Personal Protective Equipment (PPE)

Applicators and other handlers must wear:

- Long-sleeved shirt and long pants
- Shoes plus socks

Follow manufacturer's instructions for cleaning and maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

2.3 User Safety Recommendations

User should:

- Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.

2.4 Environmental Hazards

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean highwater mark. Do not contaminate water when cleaning equipment or disposing of equipment washwaters.

3.0 DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation.

4.0 AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE) and restricted-entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 4 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- Coveralls
- Waterproof gloves
- Shoes plus socks

CONTINUED

SPECIMEN

5.0 STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Store below 75°F (24°C). Keep containers tightly closed when not in use.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Disposal: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by incineration, or if allowed by state and local authorities, by burning. If burned, stay out of smoke.

6.0 PRODUCT INFORMATION

Promalin is a plant growth regulator for use on apples, non-bearing pears and non-bearing sweet cherries. When applied according to label directions to healthy, well-managed trees and under favorable weather conditions (See General Instructions), Promalin may provide one or more of the following benefits in some varieties:

1. Improve the shape of Red Delicious apples through fruit elongation and development of more prominent calyx lobes ("typiness"). These desirable effects will be more evident in years when natural typiness is limited.
2. Increase fruit size and weight on most apple varieties. Some fruit thinning may occur from the use of Promalin.
3. Increase lateral bud break and shoot growth (branching), and improve branch angle on nursery stock young trees of most apples varieties. This effect provides a better tree framework for early cropping.
4. Increase lateral bud break and shoot growth, and improve branch angle on non-bearing trees of most pear and sweet cherry varieties, including nursery stock. This effect provides a better tree framework for early cropping.

7.0 GENERAL INSTRUCTIONS

- Do not apply this product through any type of irrigation system.
- When a range of rates is indicated, use the concentration and spray volume recommended locally by the Valent agricultural specialist (1-800-6-Valent).
- For optimum effectiveness, thorough spray coverage must be achieved. All parts of the plant must receive the spray or desired results will not occur. On bearing trees, approximately 85% of the spray volume should be directed into the upper two-thirds of the trees.
- Promalin applications made under slow drying conditions (cool to warm temperatures, medium to high relative humidity, and no wind) will increase absorption by the plant, thus optimizing effectiveness. Night-time applications are encouraged when day-time conditions are not conducive to slow drying conditions.
- Do not apply Promalin when air temperatures are lower than 40°F or greater than 90°F.

CONTINUED

- For best results, the water pH should be near neutral (pH 7) and always below 8.5.
- Rainfall or overhead irrigation within 6 hours after spraying may reduce the activity of Promalin.
- **Compatibility:** The Promalin Application Instructions refer to use of the product alone. Data concerning the compatibility of Promalin with other agricultural compounds, except DiPel® DF, XenTari®, or Biobit® are not available. Valent does not assume responsibility for unexpected results due to the tank mixing of Promalin with other products.
- **Mixing Instructions:** Add the required amount of Promalin to a spray tank about half-filled with water. Agitate while bringing the total volume of water to the required level. New solutions should be mixed only in a clean, empty spray tank, and used within 24 hours. Discard any unused spray material following local, state, or federal law.

8.0 APPLICATION INSTRUCTIONS FOR FRUIT DEVELOPMENT

8.1 Rate and Timing

1. **Single Application for Improved Typiness and Size:**
   A single application of 1-2 pints of Promalin per acre should be made from early king bloom to the early stages of petal fall of the side blossoms.
2. **Split Applications for Improved Typiness and Size:**
   When the bloom period is prolonged, two applications are recommended. The first application of 0.5 to 1 pint of Promalin per acre should be made at the beginning of the bloom period, from early king bloom to the early stages of petal fall of the side bloom. The second application of 0.5 to 1 pint of Promalin per acre should be made 3 to 21 days later, or when the remainder of the canopy comes into bloom. Do not exceed the maximum recommended rate of 2 pints per acre for the combined sprays.

8.2 Spray Volume

Apply Promalin in a sufficient amount of water to ensure thorough, but not excessive, coverage. Adjust water volumes based on tree size and spacing. A suggested spray volume of 75-150 gallons/acre is adequate for most orchards. Excessive spray application volumes that result in spray runoff may reduce product performance.

9.0 APPLICATION INSTRUCTIONS FOR LATERAL BRANCHING AND TREE DEVELOPMENT

9.1 Rate and Timing

1. Application for Branching:

A single foliar application of Promalin or a Promalin-latex paint spot application may be applied to apples, non-bearing pears, and non-bearing sweet cherry trees, including nursery stock, to increase lateral bud break and shoot growth, improve branch angles, and provide a better tree framework for early cropping.

CONTINUED

Table 1 - Foliar Application

Crop	Rate	Timing
Apples (Nursery and Orchard)	125-500 ppm[1] (0.25-1 pint Promalin per 5 gallons of spray solution)[2]	For orchard trees (apples and non-bearing pears), apply at 1-3 inches of new terminal growth.
Non-bearing Pears (Nursery and Orchard)	250-1,000 ppm (0.5-2 pints Promalin per 5 gallons of spray solution)	For nursery stock (apples, non-bearing pears and non-bearing sweet cherries) treat after trees have reached a terminal height at which lateral branching is desired.
Non-bearing Sweet Cherries (Nursery only)	250-1,000 ppm (0.5-2 pints Promalin per 5 gallons of spray solution)	

[1]parts per million
[2]Do not exceed 2 pints per acre.

Table 2 - Latex Applications

Crop	Rate	Timing
Apples (Nursery and Orchard)	5,000 - 7,500 ppm[1] [0.2-0.33 pint (3.2-5.3 fluid ounces) Promalin per pint of latex paint]	Apply in the spring when terminal buds begin to swell but before shoots emerge.
Non-bearing Sweet Cherries (Orchard only)		

[1]parts per million

NOTE: Do not apply the Promalin-latex paint mixture after bud break. Applications after buds have broken may cause some injury to tender shoot tips and fail to promote shoot growth from that point.

NOTE: Uniformly apply the Promalin-latex paint mixture with a brush or sponge to cover the bark surface thoroughly. Apply only to one year old wood.

NOTE: Any type of application of Promalin to non-bearing pears and non-bearing sweet cherries must not be made later than one year prior to first anticipated fruit harvest.

10.0 NOTICE TO USER

SELLER MAKES NO WARRANTY, EXPRESS OR IMPLIED, OF MERCHANTABILITY, FITNESS OR OTHERWISE CONCERNING USE OF THIS PRODUCT OTHER THAN AS INDICATED ON THE LABEL. USER ASSUMES ALL RISKS OF USE, STORAGE OR HANDLING NOT IN STRICT ACCORDANCE WITH ACCOMPANYING DIRECTIONS.

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Libertyville, IL 60048

04-3961/R4

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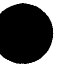

Products That Work, From People Who Care™

FOR MORE INFORMATION:
Valent U.S.A. Corporation
P.O. Box 8025
Walnut Creek, CA 94596-8025
1-800-6-VALENT - www.valent.com

2002 - PRM-0001 1/02 AV

Accel

Plant Growth Regulator
Solution

ACTIVE INGREDIENTS:

N-(phenylmethyl)-1H-purine-6-amine	1.80% w/w
Gibberellins A_4A_7	0.18% w/w
INERT INGREDIENTS	98.02% w/w
TOTAL	100.00% w/w

A plant growth regulator for use as a thinning agent on apples.
Store below 75°F (24°C).
EPA Reg. No. 73049-29
EPA Est. No. 33762-IA-001 List No. 11780

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KEEP OUT OF REACH OF CHILDREN
WARNING — AVISO
For MEDICAL and TRANSPORT Emergencies ONLY Call 24 Hours A Day 1-877-315-9819. For All Other Information Call 1-800-6-Valent.

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle. (If you do not understand the label, find someone to explain it to you in detail).

1.0 FIRST AID

If in Eyes: Hold eyelids open and flush with a steady, gentle stream of water for 15 minutes. Get medical attention.

If Swallowed: Call a doctor or get medical attention. Do not induce vomiting or give anything by mouth to an unconscious person. Drink promptly a large quantity of milk, egg whites, gelatin solution or if these are not available, drink large quantities of water. Avoid alcohol.

2.0 PRECAUTIONARY STATEMENTS

2.1 HAZARD TO HUMANS & DOMESTIC ANIMALS WARNING

Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear protective eyewear (goggles or face shield). Harmful if swallowed. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

2.2 Personal Protective Equipment (PPE)

Applicators and other handlers must wear:

- Long-sleeved shirt and long pants
- Protective eyewear
- Shoes plus socks

Follow the manufacturer's instructions for cleaning and maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

2.3 User Safety Recommendations

Users should:

- Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

2.4 Environmental Hazards

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment washwaters.

3.0 DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation.

4.0 AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE) and restricted-entry interval. The requirements in this

CONTINUED

4.0 AGRICULTURAL USE REQUIREMENTS (CONT.)

box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 12 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- Coveralls
- Waterproof gloves
- Shoes plus socks
- Protective eyewear

5.0 STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Store below 75°F (24°C). Keep containers tightly closed when not in use.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Disposal: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by incineration, or if allowed by state and local authorities, by burning. If burned, stay out of smoke.

6.0 PRODUCT INFORMATION

Accel is a plant growth regulator intended to be used as part of a comprehensive fruit thinning program. When used according to label directions on healthy, well-managed trees and under favorable weather conditions (see General Instructions), Accel may provide one or both of the following benefits in some varieties:

1. Fruit thinning
2. Increased fruit size

7.0 GENERAL INSTRUCTIONS

- Do not apply this product through any type of irrigation system.
- When a range of rates is indicated, use the concentration and spray volume recommended locally by the Valent Agricultural Specialist.
- For optimum effectiveness, thorough spray coverage must be achieved. All parts of the plant or crop must receive the spray or desired results will not occur. Approximately 85% of the spray volume should be directed into the upper two-thirds of the trees.
- Applications of Accel are most effective when ambient air temperature is around 75°F. If possible, avoid spraying Accel when ambient air temperatures are below 40°F or above 90°F. For best results, apply Accel just prior to a warming trend, or when there are likely to be 2 to 3 days of temperatures above 70°F.
- Accel applications made under slow drying conditions (medium to high relative humidity, and no wind) will increase absorption by the plant, thus optimizing effectiveness.
- Published data have shown that naphthaleneacetic acid (NAA) can cause pygmy fruit development in some varieties in some areas. Please consult your local Valent Agricultural Specialist for recommendations before including NAA and Accel in a thinning program.
- For best results, the water pH should be near neutral (pH 7) and always below 8.5.
- Rainfall or overhead irrigation within 6 hours after spraying may reduce the activity of Accel.
- **Compatibility:** The Accel spray guidelines refer to use of the product alone. Data concerning the compatibility of Accel with other agricultural compounds, except DiPel® DF, XenTari®, or Biobit® are not available. Valent does not assume responsibility for unexpected results due to the tank mixing of Accel with other products.
- **Mixing Instructions:** Add the required amount of Accel to a spray tank about half-filled with water. Agitate while bringing the total volume of water to the required level. New solutions should be mixed only in a clean, empty spray tank, and used within 24 hours. Discard any unused spray material following local, state, or federal law.

8.0 APPLICATION INSTRUCTIONS

Since many factors such as tree age, vigor, cultural practices, bloom density, and pollination, may affect fruit set and crop load, thinning programs must be based on the history of each orchard block, experience of the orchardist, and prevailing local growing conditions. Accel can be an effective component for a comprehensive thinning program with other registered materials.

One to two applications of 18 to 53.5 fluid ounces of Accel should be made between the final stages of petal fall to 10 mm average fruit diameter (Note: this period may extend from approximately 7 to 21 days after full bloom, depending on the apple variety and local weather conditions). Apply Accel under favorable weather conditions (see note on the General Instructions section).

Refer to the Rate Conversion Table for equivalency of fluid ounces of Accel and grams of active ingredient. Do not exceed 53.5 fluid ounces of Accel (30 grams of N-(phenylmethyl)-1H-purine-6-amine) per acre per application.

Accel should be applied in 50 to 200 gallons of final spray solution per acre per application. As with most plant growth regulator products, timing and total grams of active ingredient per acre are the two major factors that determine the magnitude of response. The amount of water used per acre should be maintained at a point that ensures uniform and thorough coverage of the tree canopy, but avoiding excessive wash-off of the product.

CONTINUED

9.0 RATE CONVERSION TABLE FOR ACCEL

Grams a.i.*/acre	Fluid ounces of Accel
10	18.0
15	27.0
20	35.5
25	44.5
30	53.5

**Refers to grams of N-(phenylmethyl)-1H-purine-6-amine*

10.0 NOTICE TO USER

SELLER MAKES NO WARRANTY, EXPRESS OR IMPLIED, OF MERCHANTABILITY, FITNESS OR OTHERWISE CONCERNING THE USE OF THIS PRODUCT OTHER THAN AS INDICATED ON THE LABEL. USER ASSUMES ALL RISKS OF USE, STORAGE OR HANDLING NOT IN STRICT ACCORDANCE WITH ACCOMPANYING DIRECTIONS.

Products That Work, From People Who Care

04-3312/R3 © Valent BioSciences Corporation, October 2000

Valent U.S.A. Corporation
P.O. Box 8025
Walnut Creek, CA 94596-8025
1-800-6-VALENT - www.valent.com

6-Benzyladenine

Appendix B

Regulatory information

Biopesticide Fact Sheet

Cytokinin, Kinetin, N^{6-}-Benzyladenine (116801,116802,116901)

Issued: 1/01

SUMMARY

These substances regulate plant growth and development. When applied to crops as growth enhancers, these three active ingredients are not expected to present risks to humans or the environment.

I. DESCRIPTION OF THE ACTIVE INGREDIENT

Active Ingredient Name: Cytokinin
OPP Chemical Code: 116801

Active Ingredient Name: Kinetin
OPP Chemical Code: 116802; (CAS # 525-79-1)

Active Ingredient Name: N^6-Benzyladenine
OPP Chemical Code: 116901; (CAS # 1214-39-7)

The three substances are plant growth regulators, which enhance the growth and development of plants.

Cytokinin is a mixture of four structurally-similar substances that are found naturally in plants. For commercial purposes, cytokinin is purified from seaweed meal.

Kinetin and N ^{6}–Benzyladenine are synthetic substances that are similar to cytokinin in structure and in their ability to enhance growth in plants. Note: Kinetin is sometimes found naturally as a breakdown product of nucleic acids.

II. USE SITES, USES, AND APPLICATION METHODS

Use Sites: Many food and feed crops; ornamentals.

Uses: Growth enhancer to increase yield and quality of crops.

Application Methods: Many methods for use at various stages of plant development.

III. ASSESSING RISKS TO HUMAN HEALTH

Whether or not a substance poses a risk to humans or other organisms

No adverse effects to humans are expected from use of these substances to enhance growth and maturation of crops. In tests in laboratory animals, cytokinin and kinetin showed minimal to no toxic effects. N ^{6}–Benzyladenine showed some maternal and developmental adverse

effects when it was given to pregnant rats. To minimize exposure to workers who handle large amounts of N ^{6}–benzyladenine, EPA requires that all such workers wear specified personal protective equipment (PPE).

depends on two factors: how toxic the substance is, and how much of it an organism is exposed to. Therefore, the EPA considers both toxicity and exposure data in determining whether to approve a pesticide for use

IV. ASSESSING RISKS TO THE ENVIRONMENT

No risks to the environment are expected from use of cytokinin and kinetin for reasons that include: 1) cytokinin is consumed by the many aquatic organisms that eat algae and seaweed, 2) cytokinin is used as a dietary supplement in animal feed, 3) various studies using cytokinin and kinetin found no evidence of adverse effects to birds, fish, mammals, or other wildlife.

N ^{6}–benzyladenine is slightly toxic to aquatic organisms, and consequently is not permitted to be used in or near bodies of water.

V. REGULATORY INFORMATION

Active Ingredient	Number of End Products (July 2000)	Year First Product Was Registered	Reregistration Review
Cytokinin	30	1978	1995
Kinetin	2	1995	1995
N ^{6}–Benzyladenine	8	1979	1994

VI. PRODUCER INFORMATION

Many companies have received registrations for pesticide products that contain cytokinin, kinetin, or N ^{6}–benzyladenine as an active ingredient.

VII. FOR FURTHER INFORMATION CONTACT

Brian Steinwand
Biopesticides and Pollution Prevention Division (7511C)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460
Phone: 703-305-7973 (or 308-8712)
Fax: 703-308-7026

e-mail: steinwand.brian@epa.gov

DISCLAIMER: *The information in this Bioesticide Fact Sheet is a summary only. Consult the person listed above or the Biopesticides Web Site for more information.*

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www.epa.gov/pesticides/biopesticides/factsheets/fs116801e.htm
updated April 23, 2002

United States Environmental Protection Agency | Prevention, Pesticides And Toxic Substances (7508W) | EPA-738-F-94-008 June 1994

R.E.D. FACTS

N6-Benzyladenine

Pesticide Reregistration

All pesticides sold or distributed in the United States must be registered by EPA, based on scientific studies showing that they can be used without posing unreasonable risks to people or the environment. Because of advances in scientific knowledge, the law requires that pesticides which were first registered years ago be reregistered to ensure that they meet today's more stringent standards.

In evaluating pesticides for reregistration, EPA obtains and reviews a complete set of studies from pesticide producers, describing the human health and environmental effects of each pesticide. The Agency imposes any regulatory controls that are needed to effectively manage each pesticide's risks. EPA then reregisters pesticides that can be used without posing unreasonable risks to human health or the environment.

When a pesticide is eligible for reregistration, EPA announces this and explains why in a Reregistration Eligibility Decision (RED) document. This fact sheet summarizes the information in the RED for reregistration Case 2040, N6-Benzyladenine.

Use Profile

N6-Benzyladenine is a plant growth regulator used on certain fruit and white pine trees, calla lily tubers, and spinach grown for seed. It enhances the size and shape of fruit, lateral bud break and lateral shoot growth, leading to improved branching in fruit trees and fuller white pine trees. It causes an increase in the number of calla lily flowers while decreasing time lag between first and second flowering. It also causes uniform bolting and increased seed production in spinach. N6-Benzyladenine is formulated as a soluble concentrate/liquid, and is applied using spray, brush-on and sponge-on techniques.

Regulatory History

N6-Benzyladenine was first registered as a pesticide in the U.S. in 1979. In January 1990, EPA classified it as a biochemical pesticide because it resembles natural plant growth regulators and uses a non-toxic mode of action. Currently, three products are registered and there are two Special Local Need registrations.

Human Health Assessment

Toxicity

In acute toxicity studies, N6-Benzyladenine is slightly toxic by the oral route and produces moderate eye irritation; it has been placed in Toxicity Category III (the second-to-lowest of four categories) for these effects. It is of relatively low acute dermal and inhalation toxicity, and is only a slight irritant to the skin; it has been placed in Toxicity Category IV for these effects. N6-Benzyladenine does not appear to be a skin sensitizer or mutagenic.

In a subchronic toxicity study using rats, N6-Benzyladenine caused decreased food consumption, decreased body weight gain, increased blood urea nitrogen, and minimal changes in kidney tissue. It shows some evidence of causing developmental toxicity and maternal toxicity.

Dietary Exposure

Although N6-Benzyladenine has two food crop-related uses (on fruit-bearing apple trees and spinach grown for seed), it is exempt from the requirement of a tolerance because it is a biochemical pesticide used at a rate of less than 20 grams of active ingredient per acre. Therefore, the Agency will revoke the existing tolerance and establish an exemption from the requirement of a tolerance for the currently registered uses of this pesticidal compounds on apples and spinach.

Because the use rate is low and application precedes harvest by approximately four months, the potential for dietary exposure is considered to be negligible.

Occupational and Residential Exposure

Pesticide workers (mixers, loaders and applicators) may be exposed to N6-Benzyladenine during application. Dermal exposure is expected to be moderate to high for workers who open, pour, mix and load the pesticide, and to applicators using hand sprayers and air blast equipment.

To reduce worker exposure, EPA is requiring use of the personal protective equipment (PPE) and Restricted Entry Interval set forth in the Agency's Worker Protection Standard (WPS). Because formulated products that contain N6-Benzyladenine are in Toxicity Category II, use of the following PPE is required: long-sleeved shirt and pants, socks, chemical-resistant footwear, chemical-resistant gloves, respiratory protection devices, and protective eyewear. Although the PPE requirement is based on the acute toxicity of the end-use product, it will mitigate exposure substantially and thus will serve to protect pesticide handlers from potential developmental toxicity effects. Further, the Restricted Entry Interval of 12 hours set forth in the WPS will be required, reducing the risks of post-application exposure to N6-Benzyladenine.

Human Risk Assessment

N6-Benzyladenine is of moderate to relatively low acute toxicity, but has been demonstrated to cause developmental toxicity and maternal toxicity in laboratory animals. The potential for dietary exposure is negligible. Applicator exposure and risk of developmental and maternal toxicity will be reduced through use of personal protective equipment (PPE) and the Restricted Entry Interval (REI) set forth in the Worker Protection Standard (WPS).

Environmental Assessment

Environmental Fate

Environmental fate studies were not required for N6-Benzyladenine because it is a biochemical pesticide. Soil metabolism studies indicate that it has a half-life of 7 to 9 weeks.

Ecological Effects

N6-Benzyladenine does not cause adverse effects to nontarget avian or aquatic species. It is practically nontoxic to birds, and slightly toxic to fish and freshwater invertebrates.

Ecological Effects Risk Assessment

Use of N6-Benzyladenine is not expected to pose a significant risk to terrestrial or aquatic organisms. Further, no risk to endangered species is anticipated.

Additional Data Required

EPA is requiring several generic studies as confirmatory information, including additional data for analysis of samples, a dermal sensitization study, and a mutagenicity study.

The Agency also is requiring product-specific data including product chemistry and acute toxicity studies, as well as revised Confidential Statements of Formula (CSF) and revised labeling for reregistration.

Product Labeling Changes Required

All N6-Benzyladenine end-use products must comply with EPA's current regulations and labeling requirements, and the following:

Worker Protection Standard (WPS) - All N6-Benzyladenine products within the scope of the Worker Protection Standard (WPS) for Agricultural Pesticides (see PR Notice 93-7) must, within the timeframes listed in PR Notices 93-7 and 93-11, revise their labeling to be consistent with the WPS, as directed in those notices and the requirements of the RED.

Restricted Entry Interval (REI) - The 12 hour REI set forth in the WPS is required. Labels must bear this Reentry Restriction:

- Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 12 hours.

Personal Protective Equipment (PPE) Requirements - Pesticide handlers must wear:

--coverall over short sleeved shirt and short pants;
--chemical-resistant gloves;
--chemical-resistant footwear plus socks;
--chemical-resistant headgear for overhead exposure;
--respiratory protection devices;
--protective eyewear
--chemical-resistant apron when cleaning equipment, mixing, or loading.

Regulatory Conclusion

The use of currently registered pesticide products containing N6-Benzyladenine in accordance with approved labeling will not pose unreasonable risks or adverse effects to humans or the environment. Therefore, all uses of these products are eligible for reregistration.

These products will be reregistered once the required confirmatory generic data, product specific data, Confidential Statements of Formula and revised labeling are received and accepted by EPA.

Products which contain active ingredients in addition to N6-Benzyladenine will be reregistered when all of their other active ingredients also are eligible for reregistration.

For More Information

EPA is requesting public comments on the Reregistration Eligibility Decision (RED) document for N6-Benzyladenine during a 60-day time period, as announced in a Notice of Availability published in the Federal Register. To obtain a copy of the RED document or to submit written comments, please contact the Pesticide Docket, Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs (OPP), US EPA, Washington, DC 20460, telephone 703-305-5805.

Following the comment period, the N6-Benzyladenine RED document will be available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, telephone 703-487-4650.

For more information about EPA's pesticide reregistration program, the N6-Benzyladenine RED, or reregistration of individual products containing N6-Benzyladenine, please contact the Special Review and Reregistration Division (7508W), OPP, US EPA, Washington, DC 20460, telephone 703-308-8000.

For information about the health effects of pesticides, or for assistance in recognizing and managing pesticide poisoning symptoms, please contact the National Pesticides Telecommunications Network (NPTN). Call toll-free 1-800-858-7378, from 8:00 am to 6:00 pm Central Time, Monday

through Friday.

6-benzyladenine (Accel) Tolerance Exemption 7/95

[Federal Register: July 5, 1995 (Volume 60, Number 128)]

ENVIRONMENTAL PROTECTION AGENCY
40 CFR Part 180
[OPP-300392; FRL-4963-4]
RIN 2070-AB78
6-Benzyladenine; Removal of Tolerance and Establishment of Tolerance Exemption
AGENCY: Environmental Protection Agency (EPA).
ACTION: Final rule.

SUMMARY: This document removes a tolerance for combined residues of the plant growth regulator 6-benzyladenine and establishes an exemption from the requirement of a tolerance for the chemical in or on the raw agricultural commodity apples. This document is issued in response to the Reregistration Eligibility Decision (RED) regarding this chemical and a petition from Abbott Laboratories.

EFFECTIVE DATE: This regulation becomes effective July 5, 1995.

ADDRESSES: Written objections and hearing requests, identified by the document control number, [OPP-300392], may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, Rm. M3708, 401 M St., SW., Washington, DC 20460. Fees accompanying objections and hearing requests shall be labeled "T^lerance Petition Fees" and forwarded to: EPA Headquarters Accounting C ations Branch, OPP (Tolerance Fees), P.O. Box 360277M, Pittsburgh, PA 15251. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the document control number and submitted to: Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring copy of objections and hearing requests to: Rm. 1132, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202.

A copy of objections and hearing requests filed with the Hearing Clerk may also be submitted electronically by sending electronic mail (e-mail) to: opp-docket@epamail.epa.gov. Copies of objections and hearing requests must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Copies of objections and hearing requests will also be accepted on disks in WordPerfect in 5.1 file format or ASCII file format. All copies of objections and hearing requests in electronic form must be identified by the docket number [OPP-300392]. No Confidential Business Information (CBI) should be submitted through e-mail. Electronic copies of objections and hearing requests on this rule may be filed online at many Federal Depository Libraries. Additional information on electronic submissions can be found below in this document.

FOR FURTHER INFORMATION CONTACT: By mail: Philip Poli, Special Review and Reregistration Division (7508W), Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location and telephone number: Special Review Branch, Crystal Station #1, 3rd Floor, 2800 Jefferson Davis Hwy., Arlington, VA, (703)-308-8038; e-mail: poli.philip@epamail.epa.gov.

S LEMENTARY INFORMATION: 6-Benzyladenine was first registered in the United States in 1979. It is a plant growth regulator used on certain fruit, white pine trees, calla lily tubers, and spinach grown for seed. In January 1990, the Agency classified 6-benzyladenine as a biochemical pesticide because it

resembles natural plant regulators and it displays a nontoxic mode of action. The Reregistration Eligibility Decision (RED) document was issued for 6-benzyladenine in June 1994. Based on results of acute studies that indicate low toxicity, chronic studies were not required. In addition, because the use r e is low and application precedes harvest by approximately 4 months, the p ential for dietary exposure is considered to be negligible (U.S. Environmental Protection Agency (USEPA). Reregistration Eligibility Decision (RED) document, N6-Benzyladenine, List B, Case 2040. June 1994.) The RED document proposed that the current apple tolerance be revoked and in its place an exemption from the requirement of a tolerance be established. In response to the RED, the pesticide registrant submitted a petition requesting a tolerance exemption on April 15, 1994.

EPA issued a notice, published in the Federal Register of September 28, 1994 (59 FR 49397), which announced that Abbott Laboratories had submitted a pesticide petition (PP) 4F4353 to EPA requesting that the Administrator, pursuant to section 408(d) of the Federal Food, Drug and Cosmetic Act (FFDCA), 21 U.S.C. 346a(d), amend 40 CFR part 180 to establish an exemption from the requirement of a tolerance for residues of 6-benzyladenine, N-(phenyl)-1H-purine-6-amine. No comments or requests for referral to an advisory committee were received in response to the notice. The September 28, 1994 Federal Register notice serves as the Agency's proposal to amend 40 CFR part 180 by removing the existing tolerance for apples and establishing a tolerance exemption for this chemical.

Based on the data and information considered, the Agency concludes that the tolerance exemption will protect the public health. Therefore, the tolerance exemption is established as set forth below.

Any person adversely affected by this regulation may, within 30 days after publication of this document in the Federal Register, file written objections and/or request a hearing with the Hearing Clerk, at the address given above (40 CFR 178.20). A copy of the objections and/or hearing requests filed with the Hearing Clerk should be submitted to the OPP docket for this rulemaking. T' objections submitted must specify the provisions of the regulation deemed c ectionable and the grounds for the objections (40 CFR 178.25). Each objection must be accompanied by the fee prescribed by 40 CFR 180.33(i). If a hearing is requested, the objections must include a statement of the factual issue(s) on which a hearing is requested, the requester's contentions on such issues, and a summary of any evidence relied upon by the objector (40 CFR 178.27). A request for a hearing will be granted if the Administrator determines that the material submitted shows the following: There is a genuine and substantial issue of fact; there is a reasonable possibility that available evidence identified by the requester would, if established, resolve one or more of such issues in favor of the requester, taking into account uncontested claims or facts to the contrary; and resolution of the factual issue(s) in the manner sought by the requestor would be adequate to justify the action requested (40 CFR 178.32).

A record has been established for this rulemaking under docket number [OPP-300392] (including any objections and hearing requests submitted electronically as described below). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 8 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The public record is located in Room 1132 of the Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA.

Written objections and hearing requests, identified by the document control number [OPP-300392], may be submitted to the Hearing Clerk (1900), Environmental Protection Agency, Rm. 3708, 401 M St., SW., Washington, DC 2^ 60.

A copy of electronic objections and hearing requests filed with the Hearing Clerk can be sent directly to EPA at:

opp-Docket@epamail.epa.gov

A copy of electronic objections and hearing requests filed with the

Hearing Clerk must be submitted as an ASCII file avoiding the use of special characters and any form of encryption.

The official record for this rulemaking, as well as the public version, as described above will be kept in paper form. Accordingly, EPA will transfer any o' ctions and hearing requests received electronically into printed, paper f as they are received and will place the paper copies in the official rulemaking record which will also include all objections and hearing requests submitted directly in writing. The official rulemaking record is the paper record maintained at the address in "ADDRESSES" at the beginning of this document.

Under Executive Order 12866 (58 FR 51735, Oct. 4, 1993), the Agency must determine whether the regulatory action is "significant" and therefore subject to review by the Office of Management and Budget (OMB) and the requirements of the Executive Order. Under section 3(f), the order defines a "significant regulatory action" as an action that is likely to result in a rule (1) having an annual effect on the economy of $100 million or more, or adversely and materially affecting a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or tribal governments or communities (also referred to as "economically significant"); (2) creating serious inconsistency or otherwise interfering with an action taken or planned by another agency; (3) materially altering the budgetary impacts of entitlement, grants, user fees, or loan programs or the rights and obligations of recipients thereof; or (4) raising novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in this Executive Order.

Pursuant to the terms of the Executive Order, EPA has determined that this rule is not "significant" and is therefore not subject to OMB review.

Pursuant to the requirements of the Regulatory Flexibility Act (Pub. L. 96-354, 94 Stat. 1164, 5 U.S.C. 601-612), the Administrator has determined that regulations establishing new tolerances or raising tolerance levels or establishing exemptions from tolerance requirements do not have a significant economic impact on a substantial number of small entities. A certification s ement to this effect was published in the Federal Register of May 4, 1981 (46 FR 24950).

This final rule does not contain information collection requirements subject to review by OMB under the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 et seq.

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: June 27, 1995.

Lois A. Rossi, Director, Special Review and Reregistration Division, Office of Pesticide Programs.

Therefore, 40 CFR part 180 is amended as follows:

PART 180--[AMENDED]

1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 346a and 371.

Sec. 180.376 [Removed]

2. By removing Sec. 180.376 6-Benzyladenine; tolerances for residues.

3. In subpart D, by adding new Sec. 180.1150, to read as follows:

Sec. 180.1150 6-Benzyladenine; exemption from the requirement of a tolerance.

The plant growth regulator 6-benzyladenine is exempt from the requirement of a tolerance when used as a fruit-thinning agent at an application rate not to exceed 30 grams of active ingredient per acre (30 g ai/A) in or on apples.

To Top

For more information relative to pesticides and their use, please contact the PMEP staff at:

5123 Comstock Hall
Cornell University
Ithaca, New York 14853-0901
(607)-255-1866

Last Modified: 03/01/2001
Questions regarding the development of this web site should be directed to the PMEP Webmaster.

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Disclaimer: Please read the pesticide label prior to use. The information contained at this web site is not a substitute for a pesticide label. Trade names used herein are for convenience only. No endorsement of products is intended, nor is criticism of unnamed products implied.

Notice of Proposed and Final Decisions and Directors Finding

Volume 98-45
November 09, 1998

Department of Pesticide Regulation
Pesticide Registration Branch

see page 5
N6-benzyladenine

POST THROUGH - DECEMBER 08, 1998

NOTICE OF PROPOSED DECISIONS TO REGISTER PESTICIDE PRODUCTS

The Director of the Department of Pesticide Regulation, pursuant to Section 6253 (Title 3) of the California Code of Regulations, notices the Department's following proposed decisions to register pesticide products. Interested persons may comment on these proposed decisions up to and including the date shown in the top line of this Notice to the Pesticide Registration Branch, Department of Pesticide Regulation, 830 K Street, Sacramento, California 95814-3510. Contacts regarding this notice should be made to the Pesticide Registration Branch at (916) 445-4400.

Tracking Number - EPA Registration Number
Applicant / Brand Name

172396 - (42964-17)
AIRKEM PROFESSIONAL PRODUCTS DIVISION OF ECOLAB INC.
ASEPTICARE
U DISINFECTANT - for the control of various organisms such as Staphylococcus aureus, Influenza A2 and HIV-1 on pre-cleaned environmental surfaces
Type: Section 3 label amendment - add HIV claims and remove certain use directions (air sanitizers, bacteristat and medical device precleaner decontamination uses)
Active Ingredient(s):
ETHYL ALCOHOL
ALKYL(60%C14,30%C16,5%C12,5%C18)DIMETHYL BENZYL AMMONIUM CHLORIDE
ALKYL(68%C12, 32%C14)DIMETHYL ETHYLBENZYL AMMONIUM CHLORIDE
CAS Number(s): 64-17-5 63449-41-2 8001-54-5

174016 - (7969-162)
BASF CORPORATION
BASAMID PELLETS
Use: FUNGICIDE - for protection of wood against fungal decay for commercial use only
Type: Section 3 Registration
Active Ingredient(s):
DAZOMET
CAS Number(s): 533-74-4

Notice of Proposed Decisions to Register (Continued)
Page 2

Tracking Number - EPA Registration Number
Applicant / Brand Name

172084 - (4787-23)
CHEMINOVA LTD

GLYFOS BULK
Use: HERBICIDE - for the control of weeds including fescue, bermudagrass, poorjoe, willow and bentgrass in agriculture and non-agricultural areas
T~~e: Section 3 Registration
~ ~ve Ingredient(s):
GLYPHOSATE, ISOPROPYLAMINE SALT
CAS Number(s): 38641-94-0

172853 - (6836-237)
LONZA INC. CORPORATE HEADQUARTERS
DANTOBROM RW GRANULAR
Use: ALGAECIDE/ANTI-FOULANT/SLIMICIDE - for the control of bacteria, fungi and algae in recirculating cooling water systems and sewage systems
Type: Section 3 Registration
Active Ingredient(s):
1,3-DICHLORO-5,5-DIMETHYLHYDANTOIN
1-BROMO-3-CHLORO-5,5-DIMETHYLHYDANTOIN
1,3,DICHLORO-5-ETHYL-5-METHYL HYDANTOIN
CAS Number(s): 118-52-5 126-06-7 No CAS #

172142 - (6836-107)
LONZA INC. CORPORATE HEADQUARTERS
LONZA META
Use: MOLLUSCICIDE - for manufacturing use only
Type: Section 3 label amendment - revised confidential statement of formula
Active Ingredient(s):
METALDEHYDE
(Number(s): 108-62-3

Tracking Number - EPA Registration Number
Applicant / Brand Name

172116 - (675-19)
RECKITT & COLMAN INC.
LYSOL I.C. BRAND HOSPITAL BULK DISINFECTANT CLEANER
Use: DISINFECTANT/SANITIZER - for use in institutional and industrial facilities to control Herpes Simplex type 1and Influenza Virus type A2 on hard nonporous surfaces
Type: Section 3 Registration
Active Ingredient(s):
ORTHO-PHENYLPHENOL
ORTHO-BENZYL-PARA-CHLOROPHENOL
CAS Number(s): 90-43-7 120-32-1

173288 - (3125-394)
SANTA CLARA COUNTY AGRICULTURAL COMMISSIONER
FOLICUR 3.6F FOLIAR FUNGICIDE
Use: FUNGICIDE - to control white mold, rhizoctonia limb and pod rot on peanuts
~ : Section 18 Emergency Exemption - to control garlic rust on garlic
Active Ingredient(s):
TEBUCONAZOLE
CAS Number(s): 107534-96-3

173250 - (59639-78)
VALENT U.S.A. CORPORATION
ENVOY HERBICIDE
U HERBICIDE - for the control of weeds and grasses such as foxtail, quackgrass, orchardgrass and bluegrass in conifer trees, non-bearing food crops and non crop or non-planted areas
Type: Section 3 label amendment - add weed pests, ornamentals and conifers reformat label and revise use directions
Active Ingredient(s):
CLETHODIM
CAS Number(s): No CAS #

Notice of Proposed Decisions to Register (Continued)
Page 4

Tracking Number - EPA Registration Number
Applicant / Brand Name

173265 - (707-238)
WESTERN GROWERS ASSOCIATION
CONFIRM 2F AGRICULTURAL INSECTICIDE
Use: INSECTICIDE - to control codling moth, navel orange worm and fall webworm on walnuts
Type: Section 18 Emergency Exemption - to control beet armyworm and diamondback moth in leafy vegetables and brassica leafy vegetables: cabbage, cauliflower, broccoli, spinach, head lettuce and leaf
Active Ingredient(s):
TEBUFENOZIDE
CAS Number(s): 112410-23-8

172169 - (499-405)
WHITMIRE MICRO-GEN RESEARCH LABORATORIES INC.
PRESCRIPTION TREATMENT BRAND DURAPLEX TR MICRO TOTAL RELEASE INSECTICIDE
Use: INSECTICIDE - for the control of insects including aphids, mealybugs, mites and scale in commercial garden centers and greenhouses
Type: Section 3 label amendment - added shore flies and fungus gnats
Active Ingredient(s):
CHLORPYRIFOS
CYFLUTHRIN
CAS Number(s): 2921-88-2 68359-37-5

172734 - (10182-353)
ZENECA, INC.
DIQUAT HERBCIDE
Use: HERBICIDE - for the control of weeds in alfalfa, clover, soybean, fruits and around nut trees
Type: Section 3 label amendment - revised directions for use
Active Ingredient(s):
DIQUAT DIBROMIDE
CAS Number(s): 85-00-7

Notice of Proposed Decisions to Register (Continued)
Page 5

Tracking Number - EPA Registration Number
Applicant / Brand Name

172907 - (10182-353)
ZENECA, INC.
DIQUAT HERBICIDE
U--- HERBICIDE - for the control of weeds in alfalfa, clover, soybeans, fruit and nut trees
1_ _ : Section 3 label amendment - supplemental label to add use on potatoes
Active Ingredient(s):
DIQUAT DIBROMIDE
CAS Number(s): 85-00-7

172517 - (10182-400)
ZENECA, INC.
SCIMITAR GC INSECTICIDE
Use: INSECTICIDE - for the control of various insects such as ants, armyworms, fire ants, crickets, leafhoppers and leafminers on golf courses, ornamentals and lawns
Type: Section 3 Registration
Active Ingredient(s):
LAMBDA CYHALOTHRIN
CAS Number(s): 91465-08-6

PUBLIC REPORT

SUBJECT:

Proposed registration affecting the above listed products.

DESCRIPTION OF ACTION:

The Director of Pesticide Regulation proposes to register the products listed above pursuant to Chapter 2 of Division 7 (beginning with Section 12751 of the Food and Agriculture Code) on or after December 08, 1998 . Comments concerning this proposed action may be directed to the Department of Pesticide Regulation until that date.

As a result of scientific evaluation, it has been determined that no direct or indirect significant adverse environmental impact is anticipated from the registration of the above listed products; therefore, no public report on the individual products will be filed.

ALTERNATIVES:

An effective integrated pest management strategy requires the flexibility of a large number of comparable, but not exactly equivalent, pesticides. A detailed alternatives analysis involving all anticipated crop and pest uses, under many environmental conditions and cultural practices, is beyond the scope of the normal evaluation process, when no significant adverse environmental impact which cannot be mitigated is anticipated. Such analyses are more appropriate where more specific conditions apply, such as at the user level or, in the case of restricted materials, at the level of the county agricultural commissioner. For a specified situation there may be few or no alternatives.

In the present case, in the absence of an identified significant potential adverse

impact, the benefit arising from pest management flexibility is determined to justify this registration.

Original Signed by Barry Cortez

L ..d

Barry Cortez, Chief
Pesticide Registration Branch

NOTICE OF FINAL DECISIONS TO REGISTER PESTICIDE PRODUCTS

The Director of the Department of Pesticide Regulation, pursuant to Section 6253 (Title 3) of the California Code of Regulations, files this Notice concerning pesticide products with the Secretary of the Resources Agency for posting. This Notice must remain posted for a period of 30 days for public inspection. Contacts regarding this notice should be made to the Pesticide Registration Branch at (916) 445-4400.

Tracking Number - EPA Registration Number
Applicant / Brand Name

170979 - (275-92)
ABBOTT LABORATORIES, CHEMICAL & AGRICULTURAL PRODUCTS DIV.
ACCEL PLANT GROWTH REGULATOR SOLUTION
Use: PLANT GROWTH REGULATOR - for use as a thinning agent on apples
Type: Section 3 Registration
Active Ingredient(s):
GIBBERELLINS
N' BENZYLADENINE
C.. Number(s): 77-06-5 1214-39-7

168646 - (275-104)
ABBOTT LABORATORIES, CHEMICAL & AGRICULTURAL PRODUCTS DIV.
XENTARI BIOLOGICAL INSECTICIDE DRY FLOWABLE
Use: INSECTICIDE - for the control of various insects such as loopers, armyworms, hornworms, cutworms and moths on alfalfa, berries, garlic and onions
Type: Section 3 Registration
Active Ingredient(s):
BACILLUS THURINGIENSIS (BERLINER), SUBSP. AIZAWAI, SEROTYPE H-7
CAS Number(s): No CAS #

173070 - (275-85)
ABBOTT LABORATORIES, CHEMICAL & AGRICULTURAL PRODUCTS DIV.
XENTARI WDG
Use: INSECTICIDE - for the control of various insects such as loopers, armyworms, cutworms, hornworms and webworms on crops such as grapes, strawberries, garlic, onions and alfalfa
Type: Section 3 label amendment - deletions of honey bee hazard statements
Active Ingredient(s):
BACILLUS THURINGIENSIS (BERLINER), SUBSP. AIZAWAI, SEROTYPE H-7
CAS Number(s): No CAS #

N..ce of Final Decisions to Register (Continued)
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Tracking Number - EPA Registration Number

Applicant / Brand Name

171535 - (66306-10)
AMON RE, INC.
S ARI INSECT REPELLENT SPRAY
Use: REPELLENT - to repel mosquitoes, black flies and ticks on exposed skin
Type: Section 3 Registration
Active Ingredient(s):
DEET
CAS Number(s): 134-62-3

171771 - (45017-34)
BETZ PAPERCHEM INC.
SLIME-TROL RX-68
Use: FUNGICIDE/SLIMICIDE - to control bacteria and fungi in pulp, paper mills and additive systems
Type: Section 3 Registration
Active Ingredient(s):
5-CHLORO-2-METHYL-4-ISOTHIAZOLIN-3-ONE
2-METHYL-4-ISOTHIAZOLIN-3-ONE
CAS Number(s): 26172-55-4 2682-20-4

172976 - (10163-208)
CALIFORNIA STRAWBERRY COMMISSION
SAVEY OVICIDE/MITICIDE
Use: MITICIDES - to control mites on apples
Type: Section 18 Emergency Exemption - to control mites on strawberries
Active Ingredient(s):
I :YTHIAZOX
CAS Number(s): 78-58-7

172910 - (3838-37)
ESSENTIAL INDUSTRIES, INC.
QUAT RINSE
Use: DISINFECTANT - for the control of various organisms such as E. coli, Staphylococcus aureus and Herpes Simplex Type 1 on walls, floors, sink tops, tables and chairs in hospitals
Type: Section 3 label amendment - revised directions for use
Active Ingredient(s):
ALKYL(60%C14,30%C16,5%C12,5%C18)DIMETHYL BENZYL AMMONIUM CHLORIDE
ALKYL(68%C12, 32%C14)DIMETHYL ETHYLBENZYL AMMONIUM CHLORIDE
CAS Number(s): 63449-41-2 8001-54-5

Notice of Final Decisions to Register (Continued)
Page 3

Tracking Number - EPA Registration Number
Applicant / Brand Name

166460 - (100-816)
NOVARTIS CROP PROTECTION, INC.
DUAL MAGNUM HERBICIDE
L HERBICIDE - for the control of various weeds such as barnyardgrass, goosegrass, crabgrass and pigweed in corn, cotton, peanuts, pod crops and soybeans
Type: Section 3 Registration
Active Ingredient(s):

(S)-METOLACHLOR
CAS Number(s): 87392-12-9

1⁷⁷511 - (7501-132-42056)
[illegible] .CE CHEMICALS, INC.
APRON DRY SEED PROTECTANT FUNGICIDE
Use: FUNGICIDE - for the control of soilborne diseases such as Pythium, Phytophthora and Rhizoctonia on cotton, garden beets, seed and pod vegetables and turfgrasses
Type: Section 3 Subregistration
Active Ingredient(s):
METALAXYL
CAS Number(s): 113-50-1

172809 - (400-478)
UNIROYAL CHEMICAL COMPANY, INC
B-NINE WSG
Use: PLANT GROWTH REGULATOR - to control growth of container grown ornamental plants
Type: Section 3 Registration
Active Ingredient(s):
DAMINOZIDE
CAS Number(s): 1596-84-5

Original Signed by Barry Cortez

Dated

Barry Cortez, Chief
Pesticide Registration Branch

F[illegible]T THROUGH - DECEMBER 08, 1998

NOTICE OF PROPOSED DECISIONS TO DENY PESTICIDE PRODUCTS

The Director of the Department of Pesticide Regulation, pursuant to Section 6253 (Title 3) of the California Code of Regulations, notices the Department's following proposed decisions to deny the registration of pesticide products. Unless specified, the reason for denial is that the required data was either not submitted or determined to be inadequate. Interested persons may comment on these proposed decisions up to and including the date shown on the top line of this Notice to the Pesticide Registration Branch, Department of Pesticide Regulation, 830 K Street, Sacramento, California 95814-3510.
Contacts regarding this notice should be made to the Pesticide Registration Branch at (916) 445-4400.

Tracking Number - EPA Registration Number
Applicant / Brand Name

172366 - (33677-7)
ALBRIGHT & WILSON AMERICAS
TOLCIDE PS75LT
U[illegible] ALGAECIDE/ANTIMICROBIAL - for the control of microbial contamination of oil production systems s[illegible] as water holding tanks, disposal well water and recirculating water handling systems
Type: Section 3 Registration
Active Ingredient(s):

TETRAKIS (HYDORXYMETHYL PHOSPHONIUM SULFATE
CAS Number(s): 55566-30-8

1??367 - (4564-15)
A RIGHT & WILSON AMERICAS INC
TOLICIDE PS200
Use: ANTIMICROBIAL/ALGAECIDE - for the control of bacteria, fungi and algae in the manufacture of paper and paperboard products, industrial and commercial recirculating cooling water systems, and evaporative condensers
Type: Section 3 Registration
Active Ingredient(s):
TETRAKIS (HYDORXYMETHYL PHOSPHONIUM SULFATE
CAS Number(s): 55566-30-8

Notice of Proposed Decisions to Deny (Continued)
Page 2

Tracking Number - EPA Registration Number
Applicant / Brand Name

172364 - (33677-5)
ALBRIGHT & WILSON UK LIMITED
TOLCIDE PS352C
Use: ALGAECIDE - for the control of algae, bacteria and fungi in industrial and commercial recirculating cooling water systems and evaporative condensers
Type: Section 3 Registration
Active Ingredient(s):
T RAKIS (HYDORXYMETHYL PHOSPHONIUM SULFATE
CAS Number(s): 55566-30-8

172365 - (33677-6)
ALBRIGHT & WILSON UK LIMITED 210-222 HAGLEY ROAD WEST P. O. BOX 3
TOLCIDE PS355A
Use: ALGAECIDE/ANTIMICROBIAL/BACTERICIDE - for the control of algae, bacteria, and fungi in industrial and commercial recirculating cooling water systems and evaporative condensers
Type: Section 3 Registration
Active Ingredient(s):
TETRAKIS (HYDORXYMETHYL PHOSPHONIUM SULFATE
CAS Number(s): 55566-30-8

171819 - (10951-17)
BRITZ FERTILIZERS INCORPORATED
BRITZ BT DUST
Use: INSECTICIDE - for the control of insects such as cabbage looper, cabbageworm, tobacco budworm and grape leaf folder on crops including broccoli, cabbage, celery and cotton
Type: Section 3 Registration
Active Ingredient(s):
BACILLUS THURINGIENSIS, SUBSP. KURSTAKI, STRAIN HD-1
CAS Number(s): No CAS #

N ce of Proposed Decisions to Deny (Continued)
Page 3

Tracking Number - EPA Registration Number

Applicant / Brand Name

172106 - (62719-12)
DOW AGROSCIENCES LLC
T ONE C-17
Use: FUNGICIDE/NEMATICIDE - for the control of various diseases such as black root rot, soil rot and root-knot on sweet potatoes, sugar beets and tobacco
Type: Section 3 Registration
Active Ingredient(s):
CHLOROPICRIN
1,3 DICHLOROPROPENE
CAS Number(s): 76-06-2 542-75-6

171807 - (2217-517)
PBI-GORDON CORP
TRIMEC TRUF HERBICIDE
Use: HERBICIDE - for the control of chickweed, knotweed, plantain and henbit in turf
Type: Section 3 label amendment - revised statement of practical treatment and added use on sod farms
Active Ingredient(s):
2,4-D, DIMETHYLAMINE SALT
DICAMBA, DIMETHYLAMINE SALT
MCPP, DIMETHYLAMINE SALT
CAS Number(s): 2008-39-1 2300-66-5 32351-70-5

173428 - (2217-822)
PBI/GORDON CORP.
I .355 WEED & FEED
Use: HERBICIDE - for the control of lawn weeds such as dandelions, clover, ragweed and lambquarters on bahigrass, Kentucky bluegrass, and bermudagrass
Type: Section 3 Registration
Active Ingredient(s):
DICAMBA
MCPP
MCPA
CAS Number(s): 1918-00-9 7085-19-0 794-74-6

Notice of Proposed Decisions to Deny (Continued)
Page 4

Tracking Number - EPA Registration Number
Applicant / Brand Name

172405 - (No Number Assigned)
SCRYPTON SYSTEMS INC.
BUG OIL
Use: INSECTICIDE - for the control of various insects such as aphids, gypsy moths, lace bugs, leaf miners, mealybugs, thrips and whiteflies on houseplants
Type: California Only Registration
A ve Ingredient(s):
SODIUM LAURYL SULFATE
SOYBEAN OIL
CAS Number(s): 151-21-3 8001-22-7

165527 - (8536-12)
SOIL CHEMICALS CORPORATION
MFTHYL BROMIDE 99.5%
U FUMIGANT - for space, structural and preplant soil fumigation
Type: Section 24(c) First Party Registration - for use on soil to be planted to carrots
Active Ingredient(s):
METHYL BROMIDE
CAS Number(s): 74-83-9

172783 - (499-415)
WHITMIRE MICRO-GEN RESEARCH LABS., INC.
PRESCRIPTION TREATMENT BRAND AERO-CIDE CONTACT INSECTICIDE FORMULA 1
Use: INSECTICIDE - for the control of various insects such as ants, bed bugs, clover mites, horn flies and houseflies in and around apartments, campgrounds, food storage areas, hospitals and nursing homes
Type: Section 3 Registration
Active Ingredient(s):
N-OCTYL BICYCLOHEPTENE DICARBOXIMIDE
PIPERONYL BUTOXIDE, TECHNICAL
PYRETHRINS
D-TRANS ALLETHRIN
CAS Number(s): 113-48-4 51-03-6 121-21-1 28434-00-6

Original Signed by Barry Cortez

Dated

Barry Cortez, Chief
Pesticide Registration Branch

NOTICE OF FINAL DECISIONS TO DENY PESTICIDE PRODUCTS

The Director of the Department of Pesticide Regulation, pursuant to Section 6253 (Title 3) of the California Code of Regulations, files this Notice concerning pesticide products with the Secretary of the Resources Agency for posting. This Notice must remain posted for a period of 30 days for public inspection. Unless specified, the reason for denial is that the required data was either not submitted or determined to be inadequate. Contacts regarding this notice should be made to the Pesticide Registration Branch at (916) 445-4400.

Tracking Number - EPA Registration Number
Applicant / Brand Name

171979 - (42177-44)
ALLIANCE PACKAGING CO.
E-Z CLOR MUSTARD ALGAECIDE
Use: ALGAECIDE - for the control of mustard and green algae in swimming pools
Type: Section 3 Registration
Active Ingredient(s):
COPPER SULFATE-TRIETHANOLAMINE SULFATE
CAS Number(s): 31089-39-1

1 ‚78 - (42177-44)
ALLIANCE PACKAGING CO.
REGAL ALGAEZONE PLUS

Use: ALGAECIDE - for the control of mustard and green algae in swimming pools
Type: Section 3 Registration
Active Ingredient(s):
COPPER SULFATE-TRIETHANOLAMINE SULFATE
C Number(s): 31089-39-1

Original Signed by Barry Cortez

Dated

Barry Cortez, Chief
Pesticide Registration Branch

Country	A.I.	Local Name	USName	Status	Activity	Activity Details	Date Approved	Registrant
Argentina	6-BA + GA4A7	Promalina		Filing	Re-registration			
Belgium	6-BA + GA4A7	Promalin		Approval	New Registration			
	6-BA + GA4A7	Promalin		Filing	New Registration			Abbott Belguim
Brazil	6-BA + GA4A7	Promalin		Filing	New Registration			
Canada	GA4A7+6BA	Promalin		Approval	New Registration			
Chile	6-BA + GA4A7	Promalina		Approval	Re-registration			
China	6-BA + GA4A7	Promalin		Approval	New Registration			
Czech Republic	6-BA + GA4A7	Promalin		Pending	Permit or EUP			
France	6-BA + GA4A7	Promalin		Approval	New Registration			Abbott
Israel	GA4A7+6BA	Promalin		Pending	New Registration			
Italy	6-BA + GA4A7	Promalin NT		Approval	New Registration			Abbott
	GA4A7+6BA	Promalin		Approval	New Registration			Abbott
Mexico	6-BA + GA4A7	Promalin		Approval	New Registration			
Morocco		Promalin						Marbar
New Zealand	6-BA + GA4A7	Promalin		Pending	New Registration			
Pakistan	6-BA + GA4A7	Promalin		Pending	New Registration			
Peru	6-BA + GA4A7	Promalina		Approval	New Registration			
Poland	GA4A7+6BA	Promalin 3.6 SL		Approval	New Registration			Sumi-Agro
Portugal	GA4A7+6BA	Promalin		Approval	New Registration			Abbott
Romania	GA4A7+6BA	Promalin		Pending	New Registration			Abbott
South Africa	6-BA + GA4A7	Promalin		Approval	New Registration			Abbott
South Korea	GA4A7+6BA	Promalin II		Pending	New Registration			

Spain	6-BA + GA4A7	Promalin		Approval	New Registration			Abbott
Taiwan	GA4A7+6BA	Promalin		Approval	New Registration			
Turkey	GA4A7+6BA	Promalin		Approval	New Registration			Abbott Turkey
United States	6-BA + GA4A7	Promalin		Filing	Label Extension			
	GA4A7+6BA	Promalin II		Pending	New Registration			
Uruguay	6-BA + GA4A7	Promalina		Approval	New Registration			
Zimbabwe	6-BA + GA4A7	Promalin		Filing	New Registration			

Retrieval software: DB/Text *WebPublisher*, provided by INMAGIC

Table by Country

Country	A.I.	Local Name	USName	Status	Activity	Activity Details	Date Approved	Registrant
Canada	GA4A7+6BA	Accel		Approved	Transfer Abbott to VBC			VBC
Chile	GA4A7+6BA	Accel		Approval	New Registration			
Cyprus		Accel						Stavrinides
	GA3	Accel 4% Liquid		Approval	Registration Transfer (Abbott to VBC)			Stavrinides
	GA3	Accel Tablets		Approval	Registration Transfer (Abbott to VBC)			
Egypt		Accel						Wadi El Nil
Greece		Accel						Bayer Hellas
Mexico	GA4A7+6BA	Accel		Approval	New Registration			
United States	GA4A7+6BA	Accel		Filing	New Registration			

Table by Country

Retrieval software: DB/Text *WebPublisher*, provided by INMAGIC

TOXICOLOGY DATA BASE FOR N6-BENZYLADENINE

STUDY (Species)	RESULTS	CATEGORY
Acute Oral Toxicity (rat)	LD_{50} = 1.3 grams/kg	III
Acute Dermal Toxicity (rabbit)	LD_{50} > 5 grams/kg	IV
Acute Inhalation Toxicity (rat)	LC_{50} = 5.2 mg/L	IV
Eye Irritation (rabbit)	Moderate Irritant	III
Dermal Irritation (rabbit)	Slight irritant	IV
Dermal Sensitization (guinea pig)	Not a sensitizer	N/A
Subchronic Oral Toxicity (rat)	NOEL/LOEL = 1500/5000 ppm, based on decreased BW, BW gain, food consumption	N/A
Developmental Toxicity (rat)	Maternal & Developmental NOEL/LOEL = 50/175 mg/kg/day	N/A
Mutagenicity - Ames Assay (Salmonella)	Not mutagenic	N/A
Mutagenicity - Micronucleus Assay (mouse)	Not mutagenic	N/A
Other - Unscheduled DNA Synthesis (rat hepatocytes)	Not mutagenic	N/A
Immune Response	Waived	

6-Benzyladenine

Appendix C

Physical properties, Synonyms and Material Safety Data Sheet

Data From SRC PhysProp Database:

Abbreviations in the 'Type' field: EXP = Experimental Data, EST = Estimated Data, EXT = Extrapolated Data. Extrapolated data is based upon experimental measurement outside the temperature range of the reported value. References below are abbreviated citations ... the full reference citations are NOT available here. References for Estimated data generally refer to the method used to make the estimate ... most estimates were made using SRC software.

```
CAS Number : 001214-39-7
Chem Name  : 1H-PURIN-6-AMINE, N-(PHENYLMETHYL)-
Mol Formula: C12H11N5
Mol Weight : 225.25
Melting Pt : 233 deg C
Boiling Pt :
Water Solubility:
   Value : 60 mg/L
   Temp  : 20 deg C
   Type  : EXP
   Ref   : TOMLIN,C (1997)
Log P (octanol-water):
   Value : 1.57
   Type  : EXP
   Ref   : SHAFER,WE (1990)
Vapor Pressure:
   Value : 1.79E-011 mm Hg
   Temp  : 20 deg C
   Type  : EXP
   Ref   : TOMLIN,C (1997)
pKa Dissociation Constant:
   Value :
   Temp  :
   Type  :
   Ref   :
Henry's Law Constant:
   Value : 8.84E-014 atm-m3/mole
   Temp  : 20 deg C
   Type  : EST
   Ref   : VP/WSOL
Atmospheric OH Rate Constant:
   Value : 2.14E-010 cm3/molecule-sec
   Temp  : 25 deg C
   Type  : EST
   Ref   : MEYLAN,WM & HOWARD,PH (1993)
```

Back To PhysProp Demo Page

benzyladenine

STATUS:	none
UPAC:	N^6-benzyladenine
CAS:	*N*-(phenylmethyl)-1*H*-purin-6-amine
REG. NO.:	1214-39-7
FORMULA:	$C_{12}H_{11}N_5$
ACTIVITY:	plant growth regulators (cytokinins)
NOTES:	There is no ISO common name for this substance; the name "benzyladenine" has been used in the literature but has no official status.

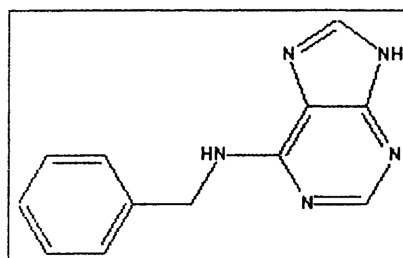
A data sheet from the Compendium of Pesticide Common Names

6-Benzylaminopurine [1214-39-7]

Synonyms: Adenine, N-benzyl-; N6-Benzyladenine; benzylaminopurine; N-(phenylmethyl)-1H-purin-6-amine; Benzyladenine; Cytokinin B; Purin-6-amine, N-(phenylmethyl)-; Verdan senescence inhibitor;

$C_{12}H_{11}N_5$

225.2524



View with ChemDraw Plugin

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VIEW CHEM3D MODEL

Add Compound Add or Change Property Add Link Feedback

Property	Value	Property	Value
ACX Number	X1006204-3	CAS RN	1214-39-7
Melting Point (°C)	230 - 233	Density	
Boiling Point (°C)		Vapor Density	
Refractive Index		Vapor Pressure	
Evaporation Rate		Water Solubility	
Flash Point (°C)		EPA Code	
DOT Number		RTECS	AU6252200
Comments			

More information about the chemical is available in these categories:

Chemical Online Order Misc Pesticides/Herbicides Physical Properties
Structures Trading

Chemical Online Order

Available Chemicals Exchange

Information about this particular compound

Misc

Chemical management
    Information about this particular compound
BIOSYNTH Biochemica & Synthetica
    Information about this particular compound

Pesticides/Herbicides

FDA Pestrak files
USEPA / OPP's Chemical Ingredients Database
    Information about this particular compound
US EPA Status of Pesticides in Registration (in PDF format)

Physical Properties

Environmental Science Center database with Experimental Log P coefficients etc.
    Information about this particular compound
ABCR GmbH&Co KG
    6-Benzyladenine, 99%
Proton NMR Spectral Molecular Formula Index
    Information about this particular compound
SPEKTR T.T.&T.

Structures

Interchem Corporation
    Information about this particular compound

Trading

Nantong ChangChem
Shanghai DSL - Supplier of Fine Chemicals and Intermediates

Enter a chemical name, CAS Number, molecular formula, or molecular weight

New Search

Substructure Query with Plug-In or Substructure Query with Java

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	ID: 001214397	CAS Number: 1214-39-7
	Enlarge Structure Use Structure For Query Use Structure for Similarity	Formula: C12-H11-N5

Names and Synonyms

Name of Substance

- N-Benzyladenine

Superlist Name

- N-(Phenylmethyl)-1H-purin-6-amine
- N6-Benzyladenine

Synonyms

- 6-(Benzylamino)purine
- 6-(N-Benzylamino)purine
- 6-BA
- 6-BAP
- 6-Benzyladenine
- ABG 3034
- Adenine, N(sup 6)-benzyl-
- Adenine, N-benzyl-
- BA
- BA (Growth stimulant)
- BAP
- BAP (growth stimulant)
- Benzyladenine
- Benzylaminopurine
- CCRIS 4351
- Caswell No. 081EE
- Cytokinin B
- EINECS 214-927-5
- EPA Pesticide Chemical Code 116901
- N(6)-Benzylaminopurine
- N(sup 6)-(Benzylamino)purine
- N(sup 6)-Benzyladenine
- N-(Phenylmethyl)-1H-purin-6-amine
- N-Benzyladenine
- N6-Benzyladenine

- NSC 40818
- Pro-Shear
- SD 4901
- SQ 4609
- Verdan senescence inhibitor

Systematic Name

- 1H-Purin-6-amine, N-(phenylmethyl)-
- Adenine, N-benzyl-
- Benzyl(purin-6-yl)amine
- Benzyladenine

Classification Codes

Classification Code

- Agricultural Chemical
- Growth regulator / Fertilizer
- Mutation data
- Plant growth regulators

Formulas

Molecular Formula

- C12-H11-N5

Locators

File Locator

- CANCERLIT
- CCRIS
- DSL
- EINECS
- EMIC
- MEDLINE
- MESH
- RTECS
- SUPERLIST
- TOXLINE
- TSCAINV

Superlist Locator

- FIFR
- INER

Registry Numbers

CAS Registry Number

- 1214-39-7

Other Registry Number

- 124786-41-0
- 3458-19-3

BORREGAARD
SYNTHESIS
MEMBER OF THE ORKLA GROUP

facsimile transmittal

To:	Sam Dolas	Fax:	925-817-5076
Company:	Valent Bio Science	Tel.	847-968-4773
From:	Brenda Quackenbush	Date:	08/06/2001
Re:	MSDS 6-Benzyladenine	Pages:	3
CC:			

☐ Urgent ☑ For Review ☐ Please Comment ☐ Please Reply ☐ Please Recycle

[1]Dear Sam:

I received a telephone request today that you are in need of the Material Safety Data Sheet for the product, 6-Benzyladeline.

Attached please find that MSDS. If I can be of further assistance please do not hesitate to contact me.

Best regards,

Brenda

[1] Direct Line: 978-463-4809
Direct Fax: 978-463-8151
Email: Brenda.Quackenbush@borregaard.com

CONFIDENTIAL

Borregaard Synthesis
9 Opportunity Way
Newburyport, MA 01950 Tel. (978) 462-5555

Material Safety Data Sheet

Revision Number: 3 Date: August 4, 1999

Section 1. Preparation \ Substance Identification

Name of Substance:	6-Benzyladenine		
Synonyms:	6-Benzylaminopurine		
CAS Number:	1214-39-7	Borregaard Stock Number:	100925
Supplier:	Borregaard Synthesis		
Address:	9 Opportunity Way		
Telephone Number:	(978) 462-5555		
Emergency Telephone Number (24 hrs):	1-978-375-5282		

State nature of emergency, Point of Contact

Section 2. Composition

Component	CAS NO.	%	PEL/TLV
6-Benzylaminopurine	1214-39-7	100	Not Established

SARA Title III Sect. 313: not listed. All ingredients on EPA TSCA Inventory. RTECS #AU6252200

Section 3. Hazard Identification

Caution: IRRITANT Contact with eye and skin may cause Irritation.
Caution: Ingestion may result in severe gastric disturbances. Gastrointestinal damage may result.
Caution: Inhalation of vapors may result in respiratory damage.

Section 4. First Aid Measures

Inhalation: Move to fresh air. Aid in breathing if necessary, and get immediate medical attention.
Eyes: Immediately wash eyes with running water for fifteen minutes. Get immediate medical attention.
Skin: Wash affected areas with water and soap. Get medical assistance if necessary.
Ingestion: If swallowed. DO NOT INDUCE VOMITING. Dilute with water or milk and get immediate medical attention. Never give fluids or Induce vomiting if the victim is unconscious or having convulsions.

Section 5. Fire Fighting Measures

Hazardous decomposition products: Emits no unusual products.
Special Fire Fighting Procedures: Use water, carbon dioxide or dry chemical extinguishing media. Firefighters should be equipped with self-contained breathing apparatus and turn out gear. Prevent build-up of static electricity inert atmospheres to control dust.
Explosion Hazards: Eliminate Explosion hazards by grounding equipment in contact with material.

Section 6. Accidental Release Measures

Personal Precautions: Use with local exhaust to control dusts. Wear an approved dust respirator, goggles, gloves, coveralls, apron, boots and other protective clothing as necessary to prevent contact.
Environmental Precautions: N/A
Clean up procedure: Spills should be contained and placed in suitable containers for disposal. This material is not regulated under RCRA or CERCLA (" Superfund").

Section 7. Handling & Storage

Handling Precautions: Use with local exhaust to control dusts. Wear an approved dust respirator, goggles, gloves, coveralls, apron, boots and other protective clothing as necessary to prevent contact.
Storage Requirement: N/A

Section 8. Exposure Controls \ Personal Protection

Occupational Exposure Limit: N/A
Biological Exposure Limit: N/A
Ventilation: Use local exhaust at point of manufacture or use.
Respiratory Protection: Approved NIOSH respirator. (Dust)
Eye Protection: Chemical goggles, Safety Glasses with side shields
Skin Protection: Gloves, coveralls, apron, boots as necessary to prevent skin contact

Section 9. Physical & Chemical Properties

Boiling/ Melting Point:	236-239 C

Appearance:	Off White to White
Odor:	Aromatic
Solubility in Water:	Insoluble
pH:	N/A
Specific Gravity:	Not established
Vapor Pressure:	N/A
Molecular Weight:	N/A

Section 10. Stability & Reactivity

Conditions to Avoid: N/A
Materials to Avoid: None known
Hazardous decomposition products: When heated to decomposition it emits nothing unusual.
Stabilization techniques: Stable

Section 11. Toxicological Information

Routes of Entry: Ingestion
Toxicity Data: LD50 Mouse >5g/kg
Acute Health Effects: N/A
Chronic Health Effects: N/A
Target Organs: N/A
IARC Cancer Review: N/A
EPA TSCA: N/A

Section 12. Ecological Information

Mobility: Not Determined
Persistence and Degradability: Not Determined
Bioaccumulative Potential: Not Determined
Aquatic Toxicity: Not Determined

Section 13. Disposal Consideration

Handling Precautions: Use with local exhaust to control dusts. Wear an approved dust respirator, goggles, gloves, coveralls, apron, boots and other protective clothing as necessary to prevent contact.
Method of Disposal: Incinerate or bury in a licensed facility. Do not discharge into waterways or sewer systems without proper authority.
Statutory Provisions:

Section 14. Transport Information

CAS Number:	1214-39-7	Packaging Group:	None
UN Number:	None	UN Class:	None
Label:	None	Special Provisions:	None

Section 15. Regulatory Information

Hazard Symbol: None
Safety Phrases: Wear proper protective gear including goggles, respirators and skin protection.

Section 16. Other Information

Recommended Uses and Restrictions: None known
Prepared by: W. Anderson Environmental Health and Safety Manager

K.H. SUNNY, INC.

2400 DALLAS STREET. HOUSTON, TEXAS 77003
TEL: 713-236-0745 FAX: 713-236-1920
kentw@khsunny.com

TO VALENT BIOSCIENCES CORPORATION
870 TECNOLOGY WAY
LIBERTYVILLE, IL 60048
TEL: 847-968-4773 /FX925-817-5076

Date: July 30, 2001

Attn: Mr. Sam Dolas, Purchase Department

Sam:

Please see attached MSDS as requested by your e-mail dated on July 30, 2001.

If there are any comments, please inform me at 713-236-0745 or my portable no: 713-299-3788

Sincerely yours,

Kent Wang

Valid 05/2001 - 07/2001

SHENGHUA BIOK CO
NO.1 ZHONGGUAN INDUSTRIAL PARK
CITY:HOUZHOU , HSIEN:DEQING ,
STATE: ZHEJIANG 313220
CHINA
TEL: 86-572-840-1658
Fax: 86-572-840-2158

MATERIAL SAFETY DATA SHEET

SECTION 1. ---------- CHEMICAL IDENTIFICATION----------

CATALOG #: PG-0100
NAME: 6-BENZYLAMINOPURINE, 99%

SECTION 2. ----- COMPOSITION/INFORMATION ON INGREDIENTS ------

CAS #: 1214-39-7
MF: C12H11N5
EC NO: 214-927-5
SYNONYMS
ABG 3034 * ADENINE, N(SUP 6)-BENZYL- * 6-BA * BA (GROWTH STIMULANT) *
6-BAP * BAP (GROWTH STIMULANT) * BENZYLADENINE * N-BENZYLADENINE *
N(SUP 6)-BENZYLADENINE * 6-BENZYLADENINE * BENZYLAMINOPURINE * N(SUP
6)-(BENZYLAMINO)PURINE * 6-(BENZYLAMINO)PURINE * 6-(N-BENZYLAMINO)
PURINE * CYTOKININ B * N-(PHENYLMETHYL)-1H-PURIN-6-AMINE * PRO-SHEAR *
1H-PURIN-6-AMINE, N-(PHENYLMETHYL)- (9CI) * SD 4901 * SQ 4609 *

SECTION 3. ---------- HAZARDS IDENTIFICATION ---------

LABEL PRECAUTIONARY STATEMENTS
IRRITANT
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING.

SECTION 4. ---------- FIRST-AID MEASURES----------

IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN. IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN. IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER. IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES.

SECTION 5. ---------- FIRE FIGHTING MEASURES ----------

EXTINGUISHING MEDIA WATER SPRAY. CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM. SPECIAL FIREFIGHTING PROCEDURES WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES. UNUSUAL FIRE AND EXPLOSIONS HAZARDS EMITS TOXIC FUMES UNDER FIRE CONDITIONS.

SECTION 6. -------- ACCIDENTAL RELEASE MEASURES---------

WEAR RESPIRATOR, CHEMICAL SAFETY GOGGLES, RUBBER BOOTS AND HEAVY RUBBER GLOVES. SWEEP UP, PLACE IN A BAG AND HOLD FOR WASTE DISPOSAL. AVOID RAISING DUST. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

SECTION 7. ----------- HANDLING AND STORAGE-----------

REFER TO SECTION 8.

SECTION 8. ------ EXPOSURE CONTROLS/PERSONAL PROTECTION------

SAFETY SHOWER AND EYE BATH. MECHANICAL EXHAUST REQUIRED. WASH THOROUGHLY AFTER HANDLING. DO NOT BREATHE DUST. AVOID CONTACT WITH EYES, SKIN AND CLOTHING. AVOID PROLONGED OR REPEATED EXPOSURE. NIOSH/MSHA-APPROVED RESPIRATOR. COMPATIBLE CHEMICAL-RESISTANT GLOVES. CHEMICAL SAFETY GOGGLES. KEEP TIGHTLY CLOSED. STORE IN A COOL DRY PLACE. IRRITANT.

SECTION 9. ------- PHYSICAL AND CHEMICAL PROPERTIES -------

APPEARANCE AND ODOR WHITE POWDER PHYSICAL PROPERTIES

MELTING POINT: 230 C TO 233 C

SECTION 10. ---------STABILITY AND REACTIVITY ---------

STABILITY STABLE.

INCOMPATIBILITIES STRONG OXIDIZING AGENTS
HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS
CARBON MONOXIDE, CARBON DIOXIDE NITROGEN OXIDES
HAZARDOUS POLYMERIZATION WILL NOT OCCUR.

SECTION 11. - - - - - - - - - TOXICOLOGICAL INFORMATION - - - - - - - -

ACUTE EFFECTS CAUSES SKIN IRRITATION.
MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN.
CAUSES EYE IRRITATION.
MAY BE HARMFUL IF INHALED.
MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT.
MAY BE HARMFUL IF SWALLOWED.
TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.
RTECS #. AU6252200
ADENINE, N-BENZYL-
TOXICITY DATA

ORL-RAT LD50:2125 MG/KG	TOIZAG 19,336,1972
ORL-MUS LD50:1300 MG/KG	TOIZAG 19,336,1972
SKN-MUS LD50:>5 GM/KG	TOIZAG 19,336,1972
SCU-MUS LD50:>2300 MG/KG	TOIZAG 19,336,1972

TARGET ORGAN DATA
SENSE ORGANS AND SPECIAL SENSES (LACRIMATION)
BEHAVIORAL (SOMNOLENCE)
ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES
(RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION.

SECTION 12. - - - - - - - - - ECOLOGICAL INFORMATION - - - - - - - - - -

DATA NOT YET AVAILABLE.

SECTION 13. - - - - - - - - - DISPOSAL CONSIDERATIONS - - - - - - - - -

DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER. OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.

SECTION 14. - - - - - - - - - - TRANSPORT INFORMATION - - - - - - - - -

CONTACT SHENGHUA BIOK COMPANY FOR TRANSPORTATION INFORMATION.

SECTION 15. - - - - - - - - - REGULATORY INFORMATION - - - - - - - - - -

EUROPEAN INFORMATION
IRRITANT
R 36/37/38
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
S 26
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
S 36
WEAR SUITABLE PROTECTIVE CLOTHING.
REVIEWS, STANDARDS, AND REGULATIONS
OEL=MAK
EPA FIFRA 1988 PESTICIDE SUBJECT TO REGISTRATION OR RE-REGISTRATION
FEREAC 54,7740,1989
EPA TSCA SECTION 8(B) CHEMICAL INVENTORY

SECTION 16. ----------- OTHER INFORMATION- -----------

THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT TO BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. SHENGHUA , KH SUNNY, INC. SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE OR

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This index contains 80,451 documents and 2,135,861 keywords.

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Display: 10 **Description:** normal **Sort:** by score

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If you have trouble with searching, you can check the following tips.

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  Namazu can't find anything with wrong spelling.
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  If you gained no results or too few results, you can add one or more related keywords with `or` operator. You should get more results. e.g.,
  `tex or ptex or latex or latex2e`
  If you gained too many results, you can add one or more related keywords with `and` operator. It makes your search more limited. e.g.,
  `latex and dvi2ps and eps`
- Try substring matching
  If you gained no results or too few results, you can try substring matching. You can specify `tex*` to search for terms which begin with `tex` (e.g., `tex`, `texi2html`, `texindex`, `text`).
  You can specify `*tex` to search for terms which terminated with `tex` (e.g., `bibtex`, `jlatex`, `latex`, `platex`, `ptex`, `vertex`).
  You can specify `*tex*` to search for terms which contain `tex` (many).
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  You can surround them respectively with double quotes like `"..."` or braces like `{...}`.

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6-Benzyladenine

Appendix D

Literature References

AGRICOLA

-Articles, etc.-

PUBLICATIONS WITH SELECTED KEYWORDS

▶ **Click** on a **number** to view the associated publication.

N6-BENZYLADENINE (29) - 29 hits		
▶1.	The effect of an elevated cytokinin level	1995
	Galis, I.	QK745 J6
▶2.	Effect of a plant-derived smoke extract,	1996,07
	Strydom, A.	QK745 P56
▶3.	Enzyme immunoassays of N6-benzyladenine	1996
	Strnad, M.	QK745 J6
▶4.	Cytokinin inhibition of Arabidopsis root	1996
	Auer, C.A.	QK745 J6
▶5.	Effects of N6-benzyladenine on shoots of	1985
	Bergman, L.	QK725 P53
▶6.	In vitro propagation of Ericaceae: a	1985,12
	Norton, M.E.	SB13 S3
▶7.	Broccoli storage: effect of	1983,11
	Shewfelt, R.L.	389.8 F7322
▶8.	Frequency of N6-benzyladenine	1984,03
	Jouanneau, J.P.	450 P692
▶9.	Incorporation of N6-benzyladenine into	1984,03
	Teyssendier de la Serve, B.	450 P692
▶10.	Broccoli storage: Effect of	1983,11
	Shewfelt, R.L.	389.8 F7322
▶11.	Comparative effects of gibberellic acid	1984
	Zack, C.D.	QK745 J6
▶12.	Effects of N6-benzyladenine and storage	1982,09
	Batal, K.M.	389.8 F7322
▶13.	Uptake and effects of N6-benzyladenine in	1982,07
	Fantelli, R.	450 P692
▶14.	Inhibition de l'incorporation de	1979,0212
	Jouanneau, J.P.	505 P21 (3)
▶15.	Increases in chlorophyll retention times	1979,10
	Blunden, G.	26 T754
▶16.	Effect of postharvest treatment with	1998
	Jiang, Y.	26 T756
▶17.	The Agrobacterium tumefaciens C58-6b gene	1999,10
	Galis, I.	450 P693

18.	Incorporation of cytokinin	1976,01
	Armstrong, D.J.	450 P692
19.	Fluorescent cytokinins: stretched-out	1976
	Sprecker, M.A.	450 P5622
20.	Comparison of N6-benzyladenine and	1976
	Thomas, T.H.	450 P564

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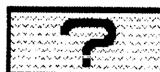
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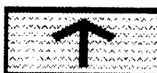
AGRICOLA
-Articles, etc.-

PUBLICATIONS WITH SELECTED KEYWORDS

▶ **Click** on a **number** to view the associated publication.

N6-BENZYLADENINE (29) - 29 hits		
▶21.	Effects of N6-benzyladenine on the rate of.	1974
	Railton, I.D.	450 P693
▶22.	Effects of N6-benzyladenine on	1975,0315
	Jeppsen, R.B.	475 EX7
▶23.	Purine ring rearrangements leading to the	1975,0820
	Leonard, N.J.	381 AM33J
▶24.	Some observations on the occurrence of	1974,09
	Jeppsen, R.B.	389.8 F7322
▶25.	Stimulation of onion bulblet production by.	1972,05
	Thomas, T.H.	80 H7892
▶26.	Flowering of Chrysanthemum under	1972,0613
	Pharis, R.P.	450 P693
▶27.	Retardation of ultraviolet light	1971,02
	Wu, J.H.	382 P56
▶28.	Incorporation of radiocarbon labelled	1969
	Matolcsy, G.	19 AC8
▶29.	IAA and N6-benzyladenine inhibit	2001,10
	Kim, J.H.	450 P699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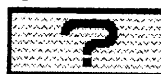
Help

AGRICOLA

-Articles, etc.-

ARTICLE RECORD

NAL CALL NO	450 P693
Author	Railton, I.D.
ArticleTitle	Effects of N6-benzyladenine on the rate of turnover of GA20 by shoots of dwarf Pisum sativum.
Other Title	Enriched title: Effects of N6-benzyladenine on the rate of turnover of [3H]GA20 [tritiated gibberellic acid] by shoots of dwarf Pisum sativum
Source Info	Planta1974, 120 (2)
Pages	p. 197-200.
Note	Includes references.

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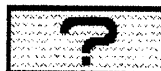
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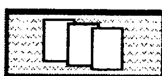
-Articles, etc.-

ARTICLE RECORD

NAL CALL NO	450 P5622
Author	Gasque, C.E.
ArticleTitle	Comparison of cytokinin activities of 9-substituted N6-benzyladenines in the Cucumis and Amaranthus bioassays Cucumis sativus, cucumbers, Amaranthus tricolor .
Source Info	Phytochemistry.1982. v. 21 (7) (ABBREV TITLE = Phytochemistry)
Pages	p. 1501-1507.
Note	38 ref.



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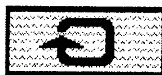

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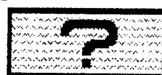

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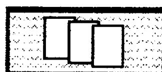
-Articles, etc.-

ARTICLE RECORD

NAL CALL NO	450 P564
Author	Thomas, T.H.
ArticleTitle	Comparison of N6-benzyladenine and N-4-pyridyl-N'-phenylurea effects on lateral bud growth, flowering and seed production of Brussels sprouts (Brassica oleracea var. gemmifera)
Source Info	1976, 38 (1) (ABBREV TITLE = Physiol Plant)
Pages	p. 35-38.
Note	Includes references.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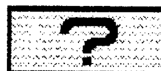
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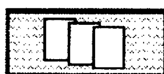
-Articles, etc.-

ARTICLE RECORD

NAL CALL NO	QK725 P53
Author	Bergman, L.
ArticleTitle	Effects of N6-benzyladenine on shoots of five willow clones (Salix spp.) cultured in vitro.
Source Info	Plant cell, tissue and organ culture. (ABBREV TITLE = Plant Cell Tissue Organ Cult) 1985. v. 4 (2)
Pages	p. 135-144.
Note	Includes 20 references.
CAB Subject	salix.
CAB Subject	clones.
CAB Subject	ba.
CAB Subject	shoot tip culture.
Other Author	Arnold, S. von.
Other Author	Eriksson, T.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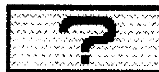
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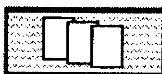
-Books, etc.-

CATALOG RECORD

NAL CALL NO	A280.39 M34Am no.537
Author	Uota, Masami, 1918-
Title	Quality and respiration rates in stock flowers treated with N6 benzylaminopurine / [by M. Uota and C.M. Harris].
Publisher	[Washington, D.C.] : U.S. Dept. of Agriculture, Agricultural Marketing Service, Market Quality Research Division, [1964].
Description	8 p. : charts ; 26 cm.
Series	AMS ; 537
Note	Cover title.
LC Subject	Flowers -- Preservation.
Other Author	Harris, C. M. (C. Max), 1934-
Other Loc	DNAL A280.39 M34Am no.537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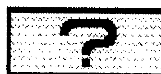
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N6-Benzyladenine
Articles on Fruit or Apple Thinning
May 8, 2002

L6 ANSWER 1 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:217968 BIOSIS
DN PREV200200217968
TI **Crop load reduction and fruit size following multi-step thinning of 'Empire' apple.**
AU Stover, Ed (1); Fargione, Mike; Risio, Richard; Yang, Xiaoe
CS (1) Indian River Research and Education Center, Univ. of Florida, 2199 South Rock Rd., Fort Pierce, FL, 34945-3138 USA
SO Hortscience, (February, 2002) Vol. 37, No. 1, pp. 130-133. print. ISSN: 0018-5345.
DT Article
LA English

L6 ANSWER 2 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 2002:28787 HCAPLUS
DN 136:146485
TI **The influence of endothal and 6-benzyladenine on crop load and fruit quality of red "delicious" apple**
AU Bound, Sally A.
CS Tasmanian Institute of Agricultural Research, New Town, 7008, Australia
SO Journal of Horticultural Science & Biotechnology (2001), 76(6), 691-699 CODEN: JHSBFA; ISSN: 1462-0316
PB Headley Brothers Ltd.
DT Journal
LA English
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:407453 BIOSIS
DN PREV200100407453
TI **Apple fruit thinning effectiveness with 6-benzyladenine and Accel.**
AU Robinson, Terence L. (1)
CS (1) Dept. of Hort. Sciences, New York State Agricultural Experiment Station, Cornell Univ., Geneva, NY, 14456 USA
SO Hortscience, (June, 2001) Vol. 36, No. 3, pp. 522. print.
Meeting Info.: 98th Annual International Conference of the American Society for Horticultural Science Sacramento, California, USA July 21-25, 2001
ISSN: 0018-5345.
DT Conference
LA English
SL English

L6 ANSWER 4 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:490466 BIOSIS
DN PREV200100490466
TI **Certain chemical thinning treatments advance maturity of Paulared apple.**
AU Embree, Charles G. (1); Nichols, Douglas S. (1); DeLong, John M. (1); Prange, Robert K. (1)
CS (1) Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main St., Kentville, NS, B4N 1J5 Canada
SO Canadian Journal of Plant Science, (July, 2001) Vol. 81, No. 3, pp. 499-501. print.
ISSN: 0008-4220.
DT Article
LA English
SL English; French

L6 ANSWER 5 OF 76 CABA COPYRIGHT 2002 CABI
AN 2002:43524 CABA
DN 20013162772
TI **Apple fruit growth responses to varying thinning methods and timing**
AU Lakso, A. N.; Robinson, T. L.; Goffinet, M. C.; White, M. D.; Palmer, J. W. [EDITOR]; Wunsche, J. N. [EDITOR]
CS NY State Agricultural Experiment Station, Department of Horticultural Sciences, Cornell University, Geneva, NY 14456, USA.
SO Acta Horticulturae, (2001) No. 557, pp. 407-412. 9 ref.
Price: EURO 84.
Meeting Info.: Proceedings of the Seventh International Symposium on Orchard and Plantation Systems, Nelson, New Zealand, 30 January-5 February 2000.
ISSN: 0567-7572; ISBN: 90-6605-8846
DT Journal; Conference Article
LA English

L6 ANSWER 6 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:437375 BIOSIS
DN PREV200000437375
TI **Studies on fruit thinning and growth in apple cultivars.**
AU Costa, Guglielmo (1); Grappadelli, Luca Corelli (1); Bucchi, Fabrizio (1)
CS (1) Dept. of Arboricolture, Bologna University, Bologna, 40126 Italy
SO Hortscience, (June, 2000) Vol. 35, No. 3, pp. 421. print.
Meeting Info.: 97th Annual International Conference of the American Society for Horticultural Science Lake Buena Vista, Florida, USA July 23-26, 2000 American Society for Horticultural Science
. ISSN: 0018-5345.
DT Conference
LA English
SL English

L6 ANSWER 7 OF 76 AGRICOLA
AN 2001:44971 AGRICOLA
DN IND23031907
TI **The use of benzyladenine in orchard fruit growing: a mini review.**
AU Buban, T.
AV DNAL (QK745.P56)
SO Plant growth regulation, Nov 2000. Vol. 32, No. 2/3. p. 381-390
Publisher: Dordrecht : Kluwer Academic Publishers.
CODEN: PGRED3; ISSN: 0167-6903
NTE In the special issue: Auxins and Cytokinins in Plant Development / edited
by E. Zazimalova, T. Thomas, D. Baker and M. Kaminek. Proceedings of an International Symposium held July 26-30, 1999, Prague, Czech Republic.
Includes references
CY Netherlands
DT Article; Law
FS Non-U.S. Imprint other than FAO
LA English

L6 ANSWER 8 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:116649 CABA
DN 20000313378
TI **Use of benzyladenine, endothall and ammonium thiosulfate for fruitlet thinning in some apple cultivars**
AU Basak, A.; Herregods, M. [EDITOR]; Boxus, P. [EDITOR]; Baets, W. [EDITOR]; Jager, A. de [EDITOR]
CS Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland.
SO Acta Horticulturae, (2000) No. 517, pp. 217-225. 13 ref.
Meeting Info.: Proceedings of the XXV International Horticultural Congress. Part 7. Quality of horticultural products: starting material, auxiliary products, quality control, Brussels, Belgium, 2-7 August, 1998.
ISSN: 0567-7572; ISBN: 90-6605-813-7
DT Conference Article; Journal
LA English

L6 ANSWER 9 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:567164 HCAPLUS
DN 135:222791
TI **The influence of Apogee and its combinations with ethephon, chemical thinners, cations, and/or adjuvants for apple tree growth control and return bloom**
AU Byers, R. E.; Carbaugh, D. H.; Combs, L. D.
CS Alson H. Smith, Jr. Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Winchester, VA, 22602, USA
SO Proceedings - Plant Growth Regulation Society of America (2000), 27th, 187-192
CODEN: PPGRDG; ISSN: 0731-1664
PB Plant Growth Regulation Society of America
DT Journal
LA English
RE.CNT 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 10 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:59454 BIOSIS
DN PREV200100059454
TI **Benzyladenine as a chemical thinner for 'McIntosh' apples. II. Effects of benzyladenine, bourse shoot tip removal, and leaf number on fruit retention.**
AU Yuan, Rongcai (1); Greene, Duane W.
CS (1) Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, 33850-2299 USA
SO Journal of the American Society for Horticultural Science, (March, 2000) Vol. 125, No. 2, pp. 177-182. print.
ISSN: 0003-1062.
DT Article
LA English
SL English

L6 ANSWER 11 OF 76 AGRICOLA
AN 2000:66429 AGRICOLA
DN IND22061564
TI **Benzyladenine as a chemical thinner for 'McIntosh' apples. I. Fruit thinning effectls and associated relationships with photosynthesis, assimilate translocation, and nonstructural carbohydrates.**
AU Yuan, R.; Greene, D.W.
AV DNAL (81 SO12)
SO Journal of the American Society for Horticultural Science, Mar 2000. Vol. 125, No. 2. p. 169-176
Publisher: Alexandria, Va. :
ISSN: 0003-1062
NTE Includes references
CY United States; Virginia
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

L6 ANSWER 12 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:464812 BIOSIS
DN PREV200000464812
TI **'McIntosh' apple fruit thinning by benzyladenine in relation to seed number and endogenous cytokinin levels in fruit and leaves.**
AU Yuan, Rongcai (1); Greene, Duane W.
CS (1) Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, 33850-2299 USA
SO Scientia Horticulturae (Amsterdam), (2 October, 2000) Vol. 86, No. 2, pp. 127-134. print.
ISSN: 0304-4238.
DT Article
LA English
SL English

L6 ANSWER 13 OF 76 AGRICOLA
AN 2000:73309 AGRICOLA
DN IND22074162
TI **'McIntosh' apple fruit thinning by benzyladenine in relation to seed number and endogenous cytokinin leaves in fruit and leaves.**
AU Yuan, R.; Greene, D.W.
AV DNAL (SB13.S3)
SO Scientia horticulturae, Oct 2, 2000. Vol. 86, No. 2. p. 127-134
Publisher: Amsterdam : Elsevier Science B.V.
CODEN: SHRTAH; ISSN: 0304-4238
NTE Includes bibliographies.
CY Netherlands
DT Article
FS Non-U.S. Imprint other than FAO
LA English

L6 ANSWER 14 OF 76 CABA COPYRIGHT 2002 CABI
AN 2001:642 CABA
DN 20000316718
TI **Net CO2 assimilation of apple leaves after the application of fruit thinning compounds**
AU Stopar, M.; Batic, F.
CS Agricultural Institute of Slovenia, Fruit and Wine Growing Department, Hacquetova 17, SI, 1001 Ljubljana, Slovenia.
SO Zbornik Biotehniske Fakultete Univerze v Ljubljani. Kmetijstvo, (2000) Vol. 75, No. 1, pp. 95-100. 21 ref.
DT Journal
LA English
SL Slovenian

L6 ANSWER 15 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:115935 CABA
DN 20000312664
TI **Effects of fruit thinning sprays of NAA and BA on cropping of 'Elstar' and 'Gloster' apples**
AU Bukovac, M. J.; Schroeder, M.; Noga, G.; Bodson, M. [EDITOR]; Verhoyen, M. N. J. [EDITOR]
CS Department of Horticulture, Michigan State University, A390 Plant and Soil Science Building, 48824 East Lansing, Michigan, USA.
SO Acta Horticulturae, (2000) No. 514, pp. 91-98. 8 ref.
Meeting Info.: Proceedings of the XXV International Horticultural Congress. Part 4. Culture techniques with special emphasis on environmental implications: chemical, physical and biological means of regulating crop growth in vegetables and fruits, Brussels, Belgium, 2-7 August, 1998.
ISSN: 0567-7572; ISBN: 90-6605-783-1
DT Conference Article; Journal
LA English

L6 ANSWER 17 OF 76 CABA COPYRIGHT 2002 CABI
AN 2001:641 CABA
DN 20000316717
TI **Comparison of the most frequently used apple thinning compounds for the thinning of 'Jonagold', 'Elstar' and 'Golden Delicious' apples**
AU Stopar, M.
CS Agricultural Institute of Slovenia, Fruit and Wine Growing Department, Hacquetova 17, SI, 1001 Ljubljana, Slovenia.
SO Zbornik Biotehniske Fakultete Univerze v Ljubljani. Kmetijstvo, (2000) Vol. 75, No. 1, pp. 89-94. 21 ref.
DT Journal
LA English
SL Slovenian

L6 ANSWER 18 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:89464 CABA
DN 20000310769
TI **Thinning apples: alternatives to carbaryl**
Diradamento del melo: le alternative al carbaryl
AU Comai, M.; Dorigoni, A.
CS Istituto agrario di S. Michele all'Adige (TN), Dipartimento produzione agricola, Unita operativa frutticoltura, Italy.
SO Informatore Agrario, (2000) Vol. 56, No. 15, pp. 89-91. 91 ref.
ISSN: 0020-0689
DT Journal
LA Italian

L6 ANSWER 19 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:276500 BIOSIS
DN PREV200000276500
TI **Developments in the chemical thinning of apple and pear.**
AU Wertheim, S. J. (1)
CS (1) Fruit Research Station, 4475 AN, Wilhelminadorp Netherlands
SO Plant Growth Regulation, (May, 2000) Vol. 31, No. 1-2, pp. 85-100. print.
ISSN: 0167-6903.
DT General Review
LA English
SL English

L6 ANSWER 20 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:115931 CABA
DN 20000312660
TI **Contributions to the efficacy of benzyladenine as a fruit thinning agent for apple cultivars**
AU Buban, T.; Lakatos, T.; Bodson, M. [EDITOR]; Verhoyen, M. N. J. [EDITOR]
CS Research and Extension Centre for Fruitgrowing, Vadastag 2, 4244 Ujfeherto, Hungary.
SO Acta Horticulturae, (2000) No. 514, pp. 59-67. 18 ref.
Meeting Info.: Proceedings of the XXV International Horticultural Congress. Part 4. Culture techniques with special emphasis on environmental implications: chemical, physical and biological means of regulating crop growth in vegetables and fruits, Brussels, Belgium, 2-7 August, 1998.
ISSN: 0567-7572; ISBN: 90-6605-783-1
DT Conference Article; Journal
LA English

L6 ANSWER 21 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:753894 HCAPLUS
DN 134:14256
TI **Contributions to the efficacy of benzyladenine as a fruit thinning agent for apple cultivars**
AU Buban, T.; Lakatos, T.
CS Research and Extension Centre for Fruitgrowing, Ujfeherto, 4244, Hung.
SO Acta Horticulturae (2000), 514(Proceedings of the XXV International Horticultural Congress, 1998, Pt. 4), 59-67
CODEN: AHORA2; ISSN: 0567-7572
PB International Society for Horticultural Science
DT Journal
LA English
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6 ANSWER 22 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:53422 CABA
DN 20000308665
TI **Testing of new fruit-thinning compounds on apples**
Preskusanje novih sredstev za redcenje plodicev jablane
AU Stopar, M.
CS Kmetijski Institut Slovenije, Oddelek za sadjarstvo in vinogradnistvo, Hacquetova 17, 1000 Ljubljana, Slovenia.
SO Sodobno Kmetijstvo, (2000) Vol. 33, No. 2, pp. 51-54. 16 ref.
DT Journal
LA Slovenian
SL English

L6 ANSWER 23 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:185217 BIOSIS
DN PREV200100185217
TI **Effect of benzyladenine on fruit set and nutrient partitioning of "Empire" apple trees.**
AU Emongor, V. E. (1); Murr, D. P.
CS (1) Crop Science Department, University of Nairobi, Nairobi Kenya
SO Discovery and Innovation, (June, 2000) Vol. 12, No. 1-2, pp. 37-43. print.
ISSN: 1015-079X.
DT Article
LA English
SL English; French

L6 ANSWER 24 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:116801 CABA
DN 20000313530
TI **Chemical thinning of apple (Malus pumila Mill.) fruits cvs Starkrimson and Golden Spur in high density plantings**
Aclareo quimico de frutos de manzano (Malus pumila Mill.) cvs. Starkrimson y Golden Spur en plantaciones de alta densidad
AU Nieto-Angel, R.; Gil-Albert Velarde, F.
CS Departamento de Fitotecnia, Universidad Autonoma Chapingo, 56230, Chapingo, Mexico.
SO ITEA Produccion Vegetal, (2000) Vol. 96, No. 1, pp. 27-41. 25 ref.
ISSN: 1130-6017
DT Journal
LA Spanish
SL English

L6 ANSWER 25 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:77332 CABA
DN 20000310557
TI **Significance of flower and fruit thinning on fruit quality**
AU Link, H.; Bangerth, F. [EDITOR]; Quinlan, J. [EDITOR]
CS Universitat Hohenheim, Institut fur Obst-, Gemuse- und Weinbau, Fachgebiet Obstbau, Bavendorf, 88213 Ravensburg, Germany.
SO Plant Growth Regulation, (2000) Vol. 31, No. 1/2, pp. 17-26. 42 ref.
Meeting Info.: Special issue: Abscission and thinning of young fruit - from molecular to applied aspects.
ISSN: 0167-6903
DT Journal
LA English

L6 ANSWER 26 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:77331 CABA
DN 20000310556
TI **The history of fruit thinning**
AU Dennis, F. G., Jr.; Bangerth, F. [EDITOR]; Quinlan, J. [EDITOR]
CS Department of Horticulture, Michigan State University, E. Lansing, MI 48824-1325, USA.
SO Plant Growth Regulation, (2000) Vol. 31, No. 1/2, pp. 1-16. 159 ref.
Meeting Info.: Special issue: Abscission and thinning of young fruit - from molecular to applied aspects.
ISSN: 0167-6903
DT Journal
LA English

L6 ANSWER 27 OF 76 CABA COPYRIGHT 2002 CABI
AN 1999:156067 CABA
DN 990311990
TI **Influence of fruit thinning chemicals on photosynthetic activity and vitality of apple leaves (Malus domestica Borkh.)**
AU Sisko, M.; Stopar, M.; Sircelj, H.; Batic, F.
CS Faculty of Agriculture, Vrbanska 30, SI-2000 Maribor, Slovenia.
SO Zbornik Biotehniske Fakultete Univerze v Ljubljani. Kmetijstvo, (1999) No. 73, pp. 135-140. 9 ref.
DT Journal
LA English
SL Slovenian

L6 ANSWER 28 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:241945 BIOSIS
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TI **Benzyladenine: its utility in regulating growth and productivity of apple trees.**
AU Buban, T. (1)
CS (1) Research and Extension Centre for Fruit Growing, H-4244, Ujfeherto Hungary
SO Biologia Plantarum (Prague), (1999) Vol. 42, No. SUPPL., pp. S82.
Meeting Info.: International Symposium on Auxins and Cytokinins in Plant Development. Prague, Czech Republic July 26-30, 1999 Institute of Experimental Botany, Academy of Sciences of the Czech Republic
. ISSN: 0006-3134.
DT Conference
LA English
SL English

L6 ANSWER 29 OF 76 CABA COPYRIGHT 2002 CABI
AN 1999:115887 CABA
DN 990308220
TI **The storage quality of apples after fruitlets thinning**
AU Basak, A.; Michalczuk, L. [EDITOR]
CS Research Institute of Pomology and Floriculture, 96-100 Skierniewice, Poland.
SO Acta Horticulturae, (1999) No. 485, pp. 47-53. 10 ref.
Meeting Info.: Proceedings of the international symposium on effect of preharvest and postharvest factors on storage of fruit, Warsaw, Poland, 3-7 August, 1997.
ISSN: 0567-7572; ISBN: 90-6605-851-X
DT Conference Article; Journal
LA English

L6 ANSWER 31 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:422 CABA
DN 990312680
TI **A study on the effect of ethephon and benzyladenine on fruit thinning in apple cultivar Gala**
Ucinek delovanja etefona in benziladenina na redcenje plodicev jablane sorte "Gala"
AU Stopar, M.
CS Kmetijski Institut Slovenije, Hacquetova 17, 1000 Ljubljana, Slovenia.
SO SAD, Revija za Sadjarstvo, Vinogradnistvo in Vinarstvo, (1999) Vol. 10, No. 9, pp. 13-16. 12 ref.
DT Journal
LA Slovenian

L6 ANSWER 32 OF 76 CABA COPYRIGHT 2002 CABI
AN 2000:409 CABA
DN 990312667
TI **Action of NAA and BA on fruit thinning in apple cultivar Golden Delicious**
Delovanje NAA in **BA** na redcenje plodicev jablane sorte "Zlati delises"
AU Stopar, M.
CS Kmetijski Institut Slovenije, Hacquetova 17, 1000 Ljubljana, Slovenia.
SO SAD, Revija za Sadjarstvo, Vinogradnistvo in Vinarstvo, (1999) Vol. 10, No. 7/8, pp. 10-12. 11 ref.
DT Journal
LA Slovenian

L6 ANSWER 33 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:135723 HCAPLUS
DN 130:149821
TI **Effect of benzyladenine on fruit thinning and its mode of action on 'McIntosh' apples**
AU Yuan, Rongcai
CS Univ. of Massachusetts, Amherst, MA, USA
SO (1998) 92 pp. Avail.: UMI, Order No. DA9841934
From: Diss. Abstr. Int., B 1999, 59(7), 3133
DT Dissertation
LA English

L6 ANSWER 34 OF 76 CABA COPYRIGHT 2002 CABI
AN 1999:71032 CABA
DN 990306125
TI **Introduction of new environmentally friendly apple thinning compounds**
Uvajanje novih, okolju prijaznejsih sredstev za redcenje plodicev jablane
AU Stopar, M.
CS Kmetijski institut Slovenije, Hacquetova 17, Ljubljana, Slovenija.
SO Agriculture and environment, proceedings, Bled, Slovenia, 12-13 March 1998, (1998) pp. 415-421. 14 ref.
Publisher: Kmetijski Institut Slovenije. Ljubljana, Slovenia
Meeting Info.: Agriculture and environment, proceedings, Bled, Slovenia, 12-13 March 1998.
ISBN: 961-6224-16-6
DT Conference Article
LA Slovenian
SL English

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AN 1998:414039 BIOSIS
DN PREV199800414039
TI **Effect of benzuladenine on apple fruit thinning and its mode of action.**
AU Yuan, Rongcai; Greene, Duane W.
CS Dep. Plant Soil Sci., Univ. Mass., Amherst, MA 01003 USA
SO Hortscience, (April, 1998) Vol. 33, No. 2, pp. 209.
Meeting Info.: Annual Meeting of the American Society of Horticultural Science (Northeast Region) Amherst, Massachusetts, USA January 9-10, 1998 American Society of Horticultural Science. ISSN: 0018-5345.
DT Conference
LA English

L6 ANSWER 36 OF 76 CABA COPYRIGHT 2002 CABI
AN 1999:70254 CABA
DN 990304986
TI **Effect of chemical fruit thinning in apple cultivars 'Fuji', 'Royal Gala' and 'Red Delicious'**
Efecto del aclareo quimico de frutos en cultivares de manzanos 'Fuji', 'Royal Gala' y 'Red Delicious'
AU Dussi, M. C.; Sanchez, E.; Veronesi, A.
CS Estacion Experimental Agropecuaria, INTA Alto Valle, CC782, (8332), General Roca, Rio Negro, Argentina.
SO ITEA Produccion Vegetal, (1998) Vol. 94, No. 3, pp. 138-147. 17 ref.
ISSN: 1130-6017
DT Journal
LA Spanish
SL English

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AN 1998:228527 BIOSIS
DN PREV199800228527
TI **Plant bioregulators in fruit production: An overview and outlook.**
AU Looney, Norman E. (1)
CS (1) Pacific Agri-Food Res. Cent., Summerland, BC V0H 1Z0 Canada
SO Journal of the Korean Society for Horticultural Science, (Feb., 1998) Vol. 39, No. 1, pp. 125-128.
ISSN: 0253-6498.
DT Article
LA English

L6 ANSWER 38 OF 76 CABA COPYRIGHT 2002 CABI
AN 1998:131169 CABA
DN 980308579
TI **Research in Europe on a new fruit thinning agent for apple trees**
V Evropi se proucujejo nova sredstva za redcenje plodicev jablane
AU Stopar, M.
CS Kmetijski Institut Slovenije, Slovenia.
SO SAD, Revija za Sadjarstvo, Vinogradnistvo in Vinarstvo, (1998) Vol. 9, No. 5, pp. 6-11. 14 ref.
DT Journal
LA Slovenian

L6 ANSWER 39 OF 76 CABA COPYRIGHT 2002 CABI
AN 1998:97142 CABA
DN 980306650
TI **Citrus production in Japan: new trends in technology**
AU Iwagaki, I.
CS Faculty of Agriculture, Shizuoka University Oh-ya 836, Shizuoka City, 422 Japan.
SO Extension Bulletin - ASPAC, Food & Fertilizer Technology Center, (1997) No. 440, pp. 11. 2 pl., 1 fig. 6 ref.
Publisher: Food and Fertilizer Technology Center for the Asian and Pacific Region. Taipei
ISSN: 0379-7597
CY Taiwan, Province of China
DT Miscellaneous
LA English
SL Chinese; Japanese; Korean

L6 ANSWER 40 OF 76 AGRICOLA
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DN IND21234515
TI **Carbaryl as a component in integrated crop management of apple.**
AU Straub, R.W.; Stover, E.; Jentsch, P.J.
AV DNAL (421 J822)
SO Journal of economic entomology, Oct 1997. Vol. 90, No. 5. p. 1315-1323
Publisher: Lanham, Md. : Entomological Society of America, 1908-
CODEN: JEENAI; ISSN: 0022-0493
NTE Includes references
CY Maryland; United States
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

L6 ANSWER 41 OF 76 AGRICOLA DUPLICATE 7
AN 1998:26808 AGRICOLA
DN IND20626939
TI **The effect of NAA and BA on carbon dioxide assimilation by shoot leaves of spur-type 'Delicious' and 'Empire' apple trees.**
AU Stopar, M.; Black, B.L.; Bukovac, M.J.
SO Journal of the American Society for Horticultural Science, Nov 1997. Vol. 122, No. 6. p. 837-840 Publisher: Alexandria, Va. : ISSN: 0003-1062
NTE Includes references
CY United States; Virginia
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

L6 ANSWER 42 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:334104 BIOSIS
DN PREV199799633307
TI **Effect of benzyladenine (BA) on fruit thinning and carbohydrate status in apples.**
AU Yuan, Rongcai; Greene, Duane W.
CS Dep. Plant Soil Sci., Bowditch Hall, Univ. Massachusetts, Amherst, MA 01003 USA
SO Hortscience, (1997) Vol. 32, No. 3, pp. 525.
Meeting Info.: 94th Annual International Conference of the American Society for Horticultural Science Salt Lake City, Utah, USA July 23-26, 1997
ISSN: 0018-5345.
DT Conference; Abstract
LA English

L6 ANSWER 43 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:334102 BIOSIS
DN PREV199799633305
TI **Effects of application conditions and adjuvants on chemical fruit thinning of apple.**
AU Stover, E. W.; Fargione, M. J.; Risio, R. A.; Mulvihill, C. I.
CS Dep. Horticultural Sci., Cornell Univ., P.O. Box 727, Highland, NY 12528 USA
SO Hortscience, (1997) Vol. 32, No. 3, pp. 524-525.
Meeting Info.: 94th Annual International Conference of the American Society for Horticultural Science Salt Lake City, Utah, USA July 23-26, 1997
ISSN: 0018-5345.
DT Conference; Abstract
LA English

L6 ANSWER 44 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:149431 HCAPLUS
DN 130:277956
TI **Benzyladenine for treating trees of hard to thin apple cultivars**
AU Buban, T.; Lakatos, T.
CS Research Station for Fruitgrowing, Ujfeherto, H-4244, Hung.
SO Acta Horticulturae (1997), 463(Eight International Symposium on Plant Bioregulators in Fruit Production, 1997), 509-515
CODEN: AHORA2; ISSN: 0567-7572
PB International Society for Horticultural Science
DT Journal
LA English
RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6 ANSWER 45 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:149429 HCAPLUS
DN 130:277954
TI **Post-bloom thinning with 6-benzyladenine**
AU Bound, S. A.; Jones, K. M.; Oakford, M. J.
CS New Town Research Laboratories, Tasmanian Institute of Agricultural Research, New Town, TAS 7008, Australia
SO Acta Horticulturae (1997), 463(Eight International Symposium on Plant Bioregulators in Fruit Production, 1997), 493-499
CODEN: AHORA2; ISSN: 0567-7572
PB International Society for Horticultural Science
DT Journal
LA English
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6 ANSWER 46 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:149425 HCAPLUS
DN 130:277923
TI **Chemical thinning of deciduous fruit trees**
AU Wertheim, S. J.
CS Research Station for Fruit Growing, Wilhelminadorp, 4475 AN, Neth.
SO Acta Horticulturae (1997), 463(Eight International Symposium on Plant Bioregulators in Fruit Production, 1997), 445-462
CODEN: AHORA2; ISSN: 0567-7572
PB International Society for Horticultural Science
DT Journal; General Review
LA English
RE.CNT 189 THERE ARE 189 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6 ANSWER 47 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:243401 HCAPLUS
DN 126:313589
TI **Integrating cytolin into a chemical thinning program for red "Delicious" apple**
AU Bound, S. A.; Jones, K. M.; Oakford, M. J.
CS New Town Research Laboratories, Tasmanian Institute of Agricultural Research, New Town, TAS. 7008, Australia
SO Aust. J. Exp. Agric. (1997), 37(1), 113-118
CODEN: AJEAEL; ISSN: 0816-1089
PB Commonwealth Scientific and Industrial Research Organization
DT Journal
LA English

L6 ANSWER 48 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:200059 HCAPLUS
DN 124:223627
TI **Thinning activity of benzyladenine on Empire apples: application, timing, and fruit storage**
AU Emongor, Vallantino Erone
CS Univ. of Guelph, Guelph, ON, Can.
SO (1996) 196 pp. Avail.: Univ. Microfilms Int., Order No. DANN00832
From: Diss. Abstr. Int., B 1996, 56(11), 5870
DT Dissertation
LA English

L6 ANSWER 49 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:484737 BIOSIS
DN PREV199598499037
TI **Thidiazuron effects on fruit set, fruit quality, and return bloom of apples.**
AU Greene, Duane W.
CS Dep. Plant Soil Sci., Univ. Mass., Amherst, MA 01003 USA
SO Hortscience, (1995) Vol. 30, No. 6, pp. 1238-1240.
ISSN: 0018-5345.
DT Article
LA English

L6 ANSWER 50 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:378224 BIOSIS
DN PREV199598392524
TI **Benzyladenine effects on cell division and cell size during apple fruit thinning.**
AU Wismer, Paul T. (1); Proctor, J. T. A.; Elfving, D. C. (1)
CS (1) Dep. Hortic. Sci., Univ. Guelph, Guelph, ON N1G 2W1 Canada
SO Hortscience, (1995) Vol. 30, No. 4, pp. 852.
Meeting Info.: 92nd Annual Meeting of the American Society for Horticultural Science and the 40th Annual Congress of the Canadian Society for Horticultural Science Montreal, Quebec, Canada July 30-August 3, 1995
ISSN: 0018-5345.
DT Conference
LA English

L6 ANSWER 51 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:409303 BIOSIS
DN PREV199598423603
TI **Benzyladenine affects cell division and cell size during apple fruit thinning.**
AU Wismer, Paul T. (1); Proctor, J. T. A. (1); Elfving, D. C.
CS (1) Dep. Hortic. Sci., Univ. Guelph, Guelph, ON N1G 2W1 Canada
SO Journal of the American Society for Horticultural Science, (1995) Vol. 120, No. 5, pp. 802-807. ISSN: 0003-1062.
DT Article
LA English

L6 ANSWER 52 OF 76 AGRICOLA
AN 97:18251 AGRICOLA
DN IND20551506
TI **Benzyladenine affects cell division and cell size during apple fruit thinning. [Erratum: Nov 1995, v. 120 (6), p. 1096.]**
AU Wismer, P.T.; Proctor, J.T.A.; Elfving, D.C.
CS University of Guelph, Guelph, Ontario, Canada.
SO Journal of the American Society for Horticultural Science, Sept 1995. Vol. 120, No. 5. p. 802-807 Publisher: Alexandria, Va. : ISSN: 0003-1062
NTE Includes references
CY United States; Virginia
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English

L6 ANSWER 54 OF 76 AGRICOLA
AN 95:63138 AGRICOLA
TI **Benyladenine and carbaryl effects on fruit thinning and the enhancement of return flowering of three apple cultivars.**
AU McArtney, S.J.; Tustin, D.S.; Seymour, S.; Cashmore, W.; Looney, N.E.
CS The Horticulture and Food Research Institute of New Zealand Ltd., Lincoln, New Zealand.
AV DNAL (80 J825)
SO The Journal of horticultural science, Mar 1995. Vol. 70, No. 2. p. 287-296
Publisher: Ashford : Headley Brothers Ltd.
CODEN: JHSCA8; ISSN: 0022-1589
NTE Includes references
CY England; United Kingdom
DT Article
FS Non-U.S. Imprint other than FAO
LA English

L6   ANSWER 55 OF 76   AGRICOLA
AN   96:15447   AGRICOLA
DN   IND20500740
TI   **Chemical fruit thinning of Vaccinium ashei Reade.**
AU   Cartagena, J.R.; Matta, F.B.; Spiers, J.M.
CS   Mississippi State University, Mississippi State, MS.
AV   DNAL (81 SO12)
SO   Journal of the American Society for Horticultural Science, Nov 1994. Vol. 119, No. 6. p. 1133-1136
Publisher: Alexandria, Va. :
ISSN: 0003-1062
NTE Includes references
CY   United States; Virginia
DT   Article
FS   U.S. Imprints not USDA, Experiment or Extension
LA   English

L6   ANSWER 56 OF 76   HCAPLUS   COPYRIGHT 2002 ACS
AN   1994:551205   HCAPLUS
DN   121:151205
TI   **Combination sprays with benzyladenine to chemically thin spur-type 'Delicious' apples**
AU   Greene, Duane W.; Autio, Wesley R.
CS   Dep. Plant Soil Sci., Univ. Massachusetts, Amherst, MA, 01003, USA
SO   HortScience (1994), 29(8), 887-90
CODEN: HJHSAR; ISSN: 0018-5345
DT   Journal
LA   English

L6   ANSWER 57 OF 76   CABA COPYRIGHT 2002 CABI
AN   94:13528   CABA
DN   940301059
TI   **Studies of the "tree factor" that inhibits the ripening of attached apples**
AU   Blanpied, G. D.
CS   Department of Fruit and Vegetable Science, Cornell University, Ithaca, New York, USA.
SO   Acta Horticulturae, (1993) No. 343, pp. 6-11. International symposium on the physiological basis of postharvest technologies, Davis, California, USA, 10-13 Aug., 1992. 14 ref.
ISSN: 0567-7572; ISBN: 90-6605-455-7
DT   Conference Article; Journal
LA   English

L6   ANSWER 58 OF 76   CABA COPYRIGHT 2002 CABI
AN   93:61111   CABA
DN   930321911
TI   **Mode of action of benzyladenine when used as a chemical thinner on apples**
AU   Greene, D. W.; Autio, W. R.; Erf, J. A.; Mao, Z. Y.
CS   Department of Plant and Soil Sciences, University of Massachusetts, Amherst, MA 01003, USA.
SO   Journal of the American Society for Horticultural Science, (1992) Vol. 117, No. 5, pp. 775-779. 38 ref.
ISSN: 0003-1062
DT   Journal
LA   English

L6 ANSWER 59 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1994:292079 HCAPLUS
DN 120:292079
TI **Laboratory and field effects of chemical fruit thinners on tetranychid and predatory mites (Acari) of apple**
AU Thistlewood, H. M. A.; Elfving, D. C.
CS Agric. Can., Vineland Res. Stn., Vineland Station, ON, LOR 2EO, Can.
SO J. Econ. Entomol. (1992), 85(2), 477-85
CODEN: JEENAI; ISSN: 0022-0493
DT Journal
LA English

L6 ANSWER 60 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1992:464754 HCAPLUS
DN 117:64754
TI **The thinning effect of benzyladenine on red Fuji apple trees**
AU Bound, Sally A.; Jones, K. M.; Koen, T. B.; Oakford, M. J.
CS Dep. Primary Ind., New Town Res. Lab., New Town, 7008, Australia
SO J. Hortic. Sci. (1991), 66(6), 789-94
CODEN: JHSCA8; ISSN: 0022-1589
DT Journal
LA English

L6 ANSWER 61 OF 76 CABA COPYRIGHT 2002 CABI
AN 92:97909 CABA
DN 920316037
TI **Effect of chemical thinning sprays on apple fruit set**
AU Byers, R. E.; Carbaugh, D. H.
CS Department of Horticulture, Agricultural Experiment Station, Virginia Polytechnic Institute and State University, Winchester, VA 22601, USA.
SO HortTechnology, (1991) Vol. 1, No. 1, pp. 41-48. 16 ref.
DT Journal
LA English

L6 ANSWER 62 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1990:454249 HCAPLUS
DN 113:54249
TI **Thinning activity of benzyladenine on several apple cultivars**
AU Greene, Duane W.; Autio, Wesley R.; Miller, Paul
CS Dep. Plant Soil Sci., Univ. Massachusetts, Amherst, MA, 01003, USA
SO J. Am. Soc. Hortic. Sci. (1990), 115(3), 394-400
CODEN: JOSHB5; ISSN: 0003-1062
DT Journal
LA English

L6 ANSWER 63 OF 76 CABA COPYRIGHT 2002 CABI
AN 91:36379 CABA
DN 910302894
TI **Effect of shade and chemical thinners on 'Redspur Delicious' fruit set**
AU Byers, R. E.
CS Agricultural Experiment Station, Winchester Virginia Polytechnic Institute and State University, Winchester, VA 22601, USA.
SO Pennsylvania Fruit News, (1990) Vol. 70, No. 2, pp. 54-59. 10 ref.
ISSN: 0031-451X
DT Journal
LA English

L6 ANSWER 64 OF 76 CABA COPYRIGHT 2002 CABI
AN 90:40836 CABA
DN 900359399
TI **Russeting of Golden Delicious apples**
AU Damas, H. F.
CS Fruit & Fruit Technology Research Institute, Stellenbosch, South Africa.
SO Deciduous Fruit Grower, (1989) Vol. 39, No. 11, pp. 421-425. 6 ref.
ISSN: 0302-7074
DT Journal
LA English

L6 ANSWER 65 OF 76 HCAPLUS COPYRIGHT 2002 ACS
AN 1990:72181 HCAPLUS
DN 112:72181
TI **The effect of some chemical agents on thinning of Golden Delicious and Granny Smith apples**
AU Lourens, A. F.
CS Fruit and Fruit Technol. Res. Inst., Stellenbosch, 7600, S. Afr.
SO S. Afr. J. Plant Soil (1989), 6(4), 223-7
CODEN: SAJSEV; ISSN: 0257-1862
DT Journal
LA English

L6 ANSWER 66 OF 76 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
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Benzyladenine and derivatives – their significance and interconversion in plants

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Abstract

Recently benzyladenine has been isolated as a natural cytokinin from a number of plants. The natural occurrence of this cytokinin will change the attitude with which physiologists view this hormone. This review attempts to put into context what is known about this cytokinin and its derivatives and to compare and contrast its metabolism and the function and physiological action of its various metabolites. Nothing is known about the biosynthesis of benzyladenine. Its structure would suggest that its biosynthetic pathway may differ considerably from that of zeatin and *iso*-pentenyladenine.

Abbreviations: Ade = adenine; Ado = adenosine; BA = benzyladenine; [9R]BA = BA ribonucleoside; [9R-MP]BA = BA nucleotide; [9R-DP]BA = BA dinucleotide; [9R-TP]BA = BA trinucleotide; [3G]BA = BA 3 glucoside; [7G]BA = BA 7 glucoside; [9G]BA = BA 9 glucoside; [9R-G]BA = BA 9-ribosylglucoside; [9Ala]BA = BA alanine-conjugate; (2OH)BA = BA ortho-OH; (2OH)[9R]BA = BA ortho-OH-riboside; KN = kinetin; [9R]KN = KN ribonucleoside; DHZ = dihydrozeatin; Z = *trans*-zeatin; [9R]Z = zeatin ribonucleoside; [7G]Z = zeatin-7-glucoside; [9G]Z = zeatin-9-glucoside; [9Ala]Z = zeatin alanine-conjugate; (OG)[9R]Z = O-glucoside of zeatin ribonucleoside; [9R-MP]Z = zeatin nucleotide; iP = *iso*-pentenyladenine; [9R]iP = iP ribonucleoside.

1. Introduction

Despite its recent identification as a naturally-occurring plant product [156] the purine cytokinin 6-(benzyl-amino)purine (Benzyladenine; BA) is still generally viewed as a synthetic compound. It is widely used in plant systems and frequently analogies are drawn between it and the synthetic, kinetin, 6-(furanosylamino)purine and naturally occurring zeatin, (6-(4-hydrozy-3-methylbut-*trans*-2-enylamino) purine), with respect to synthesis, activity, metabolism and biological activity. This approach does not necessarily give a true picture of the role of cytokinins in general in plant growth and development. This review deals specifically with BA in an attempt to get an overview of what is known about the metabolism physiology and biochemistry of this cytokinin.

Although the biochemical and physiological effects of cytokinins are well documented [121] and structure-activity patterns have emerged [132, 226], their precise action remains unknown. One prerequisite for progress in the understanding of the molecular basis of cytokinin action would seem to be a detailed knowledge of cytokinin uptake and metabolism in plant cells [47]. 'Multilevels of experimental approach' have been advocated [32] for the elucidation of the mechanism of cytokinin action. Lack of determining the active form(s) of cytokinin is probably one of the most significant unsolved problems in cytokinin research. Currently it is not known if cytokinin activity *in vivo* occurs specifically at the level of the base, riboside, or ribotide [203]. Cytokinins may not be active as such, but only after metabolic transformation into other substances [72]. Such substances may not necessarily be recognisable as cytokinins. This may explain the

limited success of hormone receptor studies to date [23].

2. The free base-benzyladenine [BA]

Benzyladenine (BA) affects the growth of both animal [15] and plant [132] cells. The base BA is an adenine derivative with a substitution on the sixth position of the purine nucleus [191]. Recently, this cytokinin was found as a free, naturally occurring cytokinin [155]. This compound has been shown to affect plant metabolism and a wide variety of physiological responses have been recorded.

Besides delaying senescence [66, 78] including both floral [216] and monocarpic senescence [128], BA promotes chlorophyll retention [181] as well as its formation [44]. Thus cytokinin enhances photosynthetic activity [1, 28, 115] and reduces respiration rates [181]. The application of BA has resulted in increased shoot to root ratios [102], increased production of ethylene [181], lowered stomatal resistance [76], increased leaf expansion [187] and stimulated protein synthesis [199]. Adverse environmental conditions have been counteracted through use of BA, including heat stress [28]. It was not shown whether this was due to BA itself or to an increase in the natural cytokinin levels. A stimulative effect of BA on plant mineral nutrition was associated with an effect on the levels of endogenous cytokinins [102].

Applied as the base, BA is currently the most frequently and most successful cytokinin used in micropropagation [204]. However, when applied to field crops, BA showed disappointing results in delaying senescence [53]. Zhang et al. [241] suggested that the 'design' of cytokinins which are more field-effective than BA 'would be facilitated by a study of the metabolism of BA'.

Morris [151] suggested that kinetin applied to roots may be converted to an endogenous cytokinin before export to the shoot. However, within elm shoots, [8-^{14}C]BA appears to be largely transported in the unmetabolised state [17]. The cytokinin ribosides are generally considered the translocatory cytokinin species [94, 229]. Despite the report on BA transport in elms, research on the potential conversion of BA to its riboside [9R]BA or to endogenous cytokinin may prove profitable.

Much circumstantial evidence derived from bioassays exists to indicate that as a base, BA is the active cytokinin form [14]. Matsubara [132] considered BA to be the most active cytokinin in the class of ring-substituted aminopurines. Cytokinin-binding protein studies [34] have more directly implicated cytokinin bases as one of the active forms. Although the base is assumed to be active *per se*, there is no unequivocal evidence to support this proposition [222, 235]. To date, this issue has not been unequivocally resolved.

Laloue and Pethe [104] presented results on growth studies with tobacco cell cultures which indicated that conversion of cytokinin ribosides to bases is necessary for activity. Uptake of exogenously supplied BA by a variety of experimental systems was mostly linear in relation to the external BA concentration, suggesting a passive role [108, 143, 227]. However, uptake of cytokinin base has also been related to the rate of cytokinin metabolism inside the cell [45, 49].

In the soybean callus bioassay [141], BA gave an optimum response when applied at a concentration between 10^{-6} and 10^{-5} *M* [213]. Activity in a similar range has been recorded for zeatin [225]. Van Staden [209] compared the activities of BA, [9R]BA and [9R-MP]BA in the same bioassay system. Of these three, BA appeared most active, with the riboside more active than the nucleotide. It was suggested that the applied cytokinins might not have been taken up by the tissue at the same rate, or that differences in the metabolism of these metabolites occurred. In this case, BA may have been taken up quicker, or metabolised more slowly if active *per se*, or quickly converted to the 'active form' of cytokinin. Problems associated with the exogenous application of cytokinins to plant systems are many and varied.

Hecht et al. [84] reported that both the nucleoside and nucleotide were less active than the corresponding base. Their findings led to the suggestion that exogenous bases do not require activation before the expression of cytokinin activity. Similarly, Laloue et al. [107] reported that iP was three times as active as its nucleoside in a tobacco callus bioassay. However, Mok et al. [150] reported that reversed activities of cytokinin bases and nucleosides were detected with iP and [9R]iP in some *Phaseolus* callus cultures.

Peters and Beck [176] reasoned that cell division-controlling substances would be expected in highest concentrations during the logarithmic phase of cell growth. Yet, at the start of the log phase in *Chenopodium* cell suspensions, free bases were detected in low concentrations, suggesting that bases may not be involved in the regulation of cell division activity. However, other researchers [85] are of the opinion that low non-polar cytokinin levels do not neces-

sarily reflect cytokinin inactivity in tissue, but rather implies their active metabolism, coupled to cytokinin action. Future research which considers endogenous cytokinin levels before, during, and after physiological responses, may yet result in a re-evaluation of the concept of 'cytokinin activity' and the molecular species associated with it.

3. Benzyladenine riboside – [9R]BA

The 9-riboside of BA ([9R]BA) is a naturally-occurring cytokinin in anise plant cells [64]. Following exogenous application of BA, [9R]BA has been identified as a prominent [54] and sometimes dominant metabolite from a variety to species [80, 134, 236]. The analogous riboside of zeatin ([9R]Z) was the main detectable metabolite when zeatin was supplied to detached leaves of *Xanthium strumarium* [87]. In *Vinca rosea* crown gall tissue, [9R]Z and the corresponding O-glucoside ((OG[9R]Z) were found by mass spectrometric technique to be the most abundant natural cytokinins [185]. Other systems have been recorded as metabolising applied BA quite differently. Conversion of BA to [9R]BA was almost negligible in radish seedlings [235].

The 9-β-D-ribonucleosides of $\underline{N}^6$-adenine derivatives have been synthesised and tested [113, 114]. Their activity in the tobacco bioassay is not as high as the corresponding base. Metabolites of BA substituted at position 9 on the purine ring were less active than the base in the soybean bioassay [74]. These authors attributed this lower activity to the difficulty which the tissues may have in converting such compounds to the base. Riboside degradation through isoprenoid sidechain cleavage (resulting in adenosine formation) has been correlated with the weak activity of [9R]iP in some tissues (*P. vulgaris* cv. Great Northern) [148]. Tissues of *P. lunatus* cv. Kingston converted the unsaturated riboside to the corresponding nucleotide, and activity was maintained. This investigation highlighted the prominent intra-specific differences in cytokinin metabolism which occurs naturally.

The free base BA was among metabolites formed from the 9-substituted cytokinin, 6-benzylamino-9-methylpurine, suggesting that the biological activity of 9-substituted cytokinins could be accounted for by their conversion to the free base [73]. Other reports confirm the relatively low activity of cytokinin ribosides, both natural and synthetic, in a variety of bioassay systems [83, 107, 118, 133, 182, 196, 209, 233]. Generally, a second substituent in the 9-position of $\underline{N}^6$-substituted purines lowers, but does not eliminate cytokinin activity [132, 192].

Not all researchers consider the riboside less active than the corresponding base. Bopp and Erichsen [22] viewed observed differences as more a consequence of restricted uptake than of an inefficiency of the substance. Peters and Beck [176] have recently considered the endogenous cytokinin patterns at all growth stages of a *Chenopodium* cell culture. They considered that cytokinin ribosides likely control cell division, more so than free bases which have traditionally been considered the active form. It remains to be determined whether activity resides in the ribonucleosides, or is acquired only on conversion to their bases. Most evidence to date has supported the latter concept [73].

Cytokinin ribosides are generally considered to be the translocatory species [163]. Trans-membrane transport of the 9-riboside of kinetin ([9R]KN) was determined by Van Staden and Mooney [224], using *Catharanthus roseus* crown gall callus. Earlier, Laloue et al. [105] had shown ready uptake of [9R]BA by tobacco cells. Movement of cytokinins within the whole plant also occurs at the riboside level. Ribosides have been detected in the xylem sap of several species, including *Urtica* [229], *Phaseolus* [179] and radish [79]. After exogenous application of BA, [9R]BA was found in senescing *Xanthium pennsylvanicum* leaves as a major product [134]. Such evidence strengthens the view [94, 121] that the riboside is a translocatory form which is exported along with other important compounds from leaves prior to senescence. Following supply of ^{14}C-BA to *Phaseolus vulgaris* plant roots, only [9R]BA was detected in xylem sap collected from the stem [179]. Similarly, the riboside was the only significant source of radioactivity in the xylem sap of radish seedling after application of ^{14}C-Z [79]. These findings indicate that translocation of cytokinins from the roots is in the riboside form. Notably, ribosides are also major cytokinins in the phloem sap [121], indicating that the transport of ribosides may also be transported from shoots to roots.

A storage function for nucleosides has also been implied. A cytokinin riboside ([9R]Z) was detected in the (storage) roots of chicory by Bui-Dang-Ha and Nitsch [25]. It was not determined whether this riboside was synthesised *in situ* or merely stored there.

4. Benzyladenine-nucleotide-[9R-MP]BA

Following application of BA, [9R-MP]BA has been identified as a metabolite of BA in *Lemna minor* [16] soybean callus [70] and in *Acer pseudoplatanus* cell cultures [47]. The monophosphate of BA is relatively stable, as shown by its metabolic half-life in tobacco cells (8 days) [105]. In other systems which initially produced cytokinin mono-nucleotide as the principal metabolite, levels rapidly became subdominant [79].

Laloue and Pethe [104] considered cytokinin riboside-5′-phosphates to play a central role in the regulation of the levels of the various metabolic forms of cytokinins as they are readily interconverted to the riboside, and to the base. A role for cytokinin mono-nucleotides in hormonal homeostasis is generally accepted [164]. The main feature of inter-conversion pathways in this active cytokinin pool is that their overall equilibrium is thought to be in favour of nucleotide formation [105], although such conversion may involve a steady state maintenance of base and riboside levels [104]. Support of this homeostatic notion is provided by the fact that when cytokinin-dependent soybean callus was fed [9R-MP]Z [221], only the corresponding base and nucleoside were produced [104]. Laloue et al. [105] claimed that nucleotide isolation and identification has been neglected. These workers proposed that more attention should be paid to ribotides as naturally occurring cytokinins with a central role. A later report by Scott and Horgan [184] which employed mass spectrometric techniques has shown that cytokinin nucleotides may be more abundant than has been previously shown. These authors demonstrated that the nucleotide is more abundant than the ribosides in tissues where this was previously seen to be otherwise. Scott and Horgan [184] predicted that the application of 'new analytical techniques for cytokinin nucleotides will result in an extensive re-evaluation of the existing cytokinin literature'. Such a re-evaluation has not yet occurred.

According to Ashihara [8], purine nucleotidase are synthesised both from i.e. the *de novo* pathway amino acids, CO_2, tetrafolate derivatives and α-5-phosphoribosyl-1-pyrophosphate (PRPP) and from preformed purine bases and their ribonucleosides (the salvage pathway). Nothing is known of BA biosynthesis in plants [99]. Should biosynthesis of 6-(benzylamino)purine proceed at the nucleotide level, as suggested for *iso*-pentenyl-type cytokinins [121, 201], then [9R-MP]BA will likely play an essential role as an intermediate precursor in those tissues where naturally-occurring BA metabolites are known to occur [197]. However, in this cytokinin there is a benzyl ring attached at the ^{6}N-position of adenine. This makes it unlikely that iso-pentenyl transferase would be involved. It seems that a common biosynthetic pathway may not exist for iP and BA cytokinins.

Once in the active pool, the nucleotide may exist *via* the base or the riboside to N-conjugates, or oxidative catabolites. [9R-MP]BA has been implicated as the immediate precursor of [7G]BA in tobacco systems [71]. Conversely, [7G]BA applied to tobacco was (indirectly) converted to BA nucleotides [77]. These authors suggested that such a conversion is indirect, via the transient formation of BA. As with the other cytokinin species which contribute to the active pool, the exact role(s) of nucleotides remains to be fully elucidated.

Nucleotides have been associated with storage of cytokinins [121, 209]. Pietraface and Blaydes [177] provided evidence to show that the nucleotide is a storage from in lettuce seeds before conversion to the active nucleoside.

Nucleotide formation may also be associated with cytokinin uptake [164] and transport across membranes, in much the same way as phosphoribosylation plays a role in the uptake of adenine by *Escherichia coli* membranes [121]. Burch and Stuchbury [27] noted that although polar cytokinins such as nucleotides are common metabolites within cells, they have rarely been identified in the culture media. This has been considered indicative of plasmalemma impermeability to [9R-MP]BA [104, 105]. However, following incubation of [9R-MP]Z with soybean callus, various zeatin metabolites were extracted from cellular contents. High levels of [9R-MP]Z detected in artichoke tissues shortly after the start of culture was cited as evidence [164] that the nucleotides are involved in cytokinin uptake. Yet, with the aid of adenine phosphoribosyltransferase-deficient (APRT) mutants, Moffatt et al. [145] have recently shown that phosphoribosylation of BA is not a prerequisite for its uptake by *Arabidopsis* plants.

A translocatory role for this cytokinin class has been proposed. Free base applied to bean roots was recovered in the stem, partly as the nucleotide [232]. Both ribosides and ribotides were identified by Palmer et al. [163] in stems of decapitated, disbudded bean plants. Vonk and Davelaar [228] suggested that the nucleotides are cytokinins in transport in the phloem. The highest levels of [9R-MP]BA detected in tomato

plants were in the stems [14], again implying a translocatory role.

Shaw et al. [189] considered the cytokinin activity of the 3-, 7-, and 9-methyl derivatives of zeatin. The results implied that the mechanism of cytokinin activity in substituted adenines does not require prior formation of nucleotide derivatives. In contrast, Laloue et al. [105] correlated division of cytokinin-requiring tobacco cells with high levels of cytokinin ribotides and low levels of cytokinin base and riboside, irrespective of whether the base or riboside was supplied. The fact that the methyl group in the 9-position does not constitute an effective block, but is easily metabolised, makes suspect the presumed stability of other 9-substituted cytokinins [73]. When compared in the tobacco callus bioassay, [9R]BA and [9R-MP]BA were less active at lower concentrations (10^{-1} μM) than BA [183]. More recently, similar results were obtained using the soybean callus bioassay [219].

The identity of the actual 'active cytokinin' form(s) remains an unresolved issue. The nucleotide may be necessary for the expression of activity [105], but if not, is likely to contribute to the steady state maintenance of an active cytokinin pool.

5. Di- and tri-phosphates of benzyladenine ([9R-DP]BA and [9R-TP]BA)

The di- and tri-nucleotides of BA have been detected in extracts of *Petunia* leaves following incubation with BA [10, 11, 12]. These metabolites were rapidly produced by the explants and were considered by these authors to be active forms responsible for shoot induction. An earlier indication of the activity of cytokinin polynucleotides was revealed by Miller [140]. This author extracted a cytokinin from *Zea mays* kernels possessing 'at least two phosphate groups', which showed some activity in a soybean callus bioassay. Bezemer-Sybrandy and Veldstra [16] detected mono-, di, and tri-nucleotides of BA as metabolites in *Lemna minor* cultures. The formation of such nucleotides was considered to be a normal feature of cytokinin metabolism in plant tissues [140, 206], and to indicate the natural occurrence of analogous endogenous nucleotides in plant tissues [106].

The existence *in vivo* of cytokinin nucleoside-5′-triphosphate is of theoretical importance as such compounds could be incorporated into RNA molecules [69, 106] to provide a basis for cytokinin action. Incorporation of BA into RNA was demonstrated by Fox [68]. Armstrong et al. [5] similarly viewed [9R-TP]BA as an important intermediate in a pathway for the incorporation of BA into RNA species. The incorporation of cytokinin bases into polynucleotides [33] indicates that the 5′-monophosphate is an intermediate metabolite in the reaction. Preferential incorporation of label into the guanine fraction of soluble RNA hydrolysates from soybean and tobacco callus cultured on media containing ^{14}C-BA was recorded by Fox [69]. In soybean callus, [9R-MP]BA appeared as the major metabolite [70]. Cytokinin-dependent tobacco callus supplied with BA incorporated this compound in low levels in both tRNA and rRNA though mainly in the rRNA [5]. Jouanneau and Teyssendier de la Serve [98] considered this to occur through a direct insertion process. Despite the large number of BA metabolic studies in plant tissues, detection of di- and tri-nucleotides has rarely been reported. When identified, these compounds are normally minor metabolites [106]. *Acer* cultures supplied with $\underline{N}^6$-substituted nucleosides did not phosphorylate these compounds beyond the monophosphate level [47, 48]. In contrast, 3 hours after application of ^{14}C-BA to tobacco cell cultures, 6% of the radioactivity was associated with [9R-DP]BA and [9R-TP]BA [106]. The monophosphate ([9R-MP]BA) represented 28%, the base 30% and [9R]BA was unrepresented. To explain differences in the metabolism of BA observed between *Acer* and tobacco, Laloue et al. [106] suggested that the cytokinin inactivation through sidechain cleavage noted for *Acer* [47] would restrict formation of the di- and tri-nucleotides.

The hydrolytic action of 5^1-nucleotidases which show equal affinity for mono-, di- and tri-phosphates of adenosine [35] are likely responsible for release of [9R]BA from [9R-DP]BA and [9R-TP]BA. Such a conversion could produce a more active cytokinin species, either in the form of [9R]BA, or after deribosylation of [9R]BA to the base.

6. N-conjugation of BA

Collectively, the N-alanyl conjugates and N-glucosides of cytokinins are referred to as N-conjugates.

N-conjugates are stable both when applied externally and when found as metabolites [170, 173] and are generally incapable of further metabolism back to base [136, 138]. For this reason N-conjugates are regarded as detoxification or inactivation products [77, 121] rather than storage forms; a role proposed for the

O-glucosides of zeatin [75]. Hence N-conjugation may result in the irreversible loss of cytokinin activity.

In reducing levels of cytokinin activity, plants may oxidise the 'active compound' [198], or alternatively glucosylate/alanylate. McGaw and Horgan [138] distinguished an 'oxidative-type' metabolism from a 'glucosidase-type' metabolism to describe either oxidative cleavage of the $\underline{N}^6$-sidechain, or conjugation of exogenously supplied cytokinin. In many tissues BA is resistant to attack by cytokinin oxidase. Consequently, N-conjugation may provide the only mechanism by which the biological activity of this cytokinin might be controlled. Side-chain cleavage of N-conjugates [168] may be a means of reducing still further the activity of these detoxification products.

7. Benzyladenine - Metabolism

Although application of a cytokinin metabolite to a bioassay system may promote an active response, each of these 'active' compounds are themselves rapidly metabolised to an extensive range of products, many of which are active to varying degrees in the same bioassay [219]. Mok et al. [146] observed that the activity of a particular cytokinin may depend on the bioassay system used. Activity differences between metabolites were attributed by these authors to reflect uptake, compartmentation, sensitivities to enzymes, or binding site specificities. In different bioassays these components may change, so altering the effectiveness of particular metabolites in inducing a response. In an alternative experimental approach, studies of endogenous cytokinin levels during different phases of plant growth [176] have provided insight into *in vivo* biological activity. the manipulation of endogenous cytokinin levels by genetic transformation has also been considered more useful in revealing natural processes than exogenous applications to isolated organs or calli [3, 82].

Plant tissues convert exogenous BA into a great diversity of metabolites which include products of ring substitution (ribosides, nucleotides, N-glucosides), and products of sidechain cleavage (e.g. adenine, adenosine, and adenosine-5′-monophosphate [121].

The functional significance of these metabolites remains obscure [229], but it has been suggested [121] that these compounds could be:

1. Active forms of cytokinin, i.e. the molecular species which bind to a receptor to evoke a growth or physiological response;
2. Translocation forms;
3. Storage forms which would release free (active) cytokinin when required;
4. Detoxification products formed following exogenous cytokinin application at toxic levels;
5. Deactivation products formed to lower endogenous (active) cytokinin levels; and
6. Postactivation products, formation of which is coupled with cytokinin action (formed as a result of cytokinin utilisation).

McGaw and Horgan [138] indicated that an understanding of compartmentation with respect to the mechanisms and sites of cytokinin action needs to occur before the exact roles of various cytokinins may be assigned. Until this knowledge is obtained and activities can be measured directly at the site of action [146] prescribed roles will remain mainly speculative.

Chen [32] considered fundamental control mechanisms to be those operating at the level of enzymic regulation of metabolism (biosynthesis, interconversion, and degradation). Several major enzymic pathways compete for cytokinins, by which they are inter-converted and degraded [32]. Burch and Stuchbury [27] noted that enzymes metabolising adenine derivatives [34, 35, 38] exhibit a low degree of specificity for the exact structure of the purine ring and hence the same enzymes will actively metabolise many $\underline{N}^6$-substituted cytokinins albeit at different rates. The fate of a cytokinin may be attributed to the relative activities of cytokinin metabolic enzymes, which in turn are affected by the relative concentrations and distribution of the hormone and its precursors in the plant cell [32]. Given the lack of specificity of some cytokinin-metabolising enzymes, metabolism of cytokinins may be limited by competition for the enzymes. Hence Burch and Stuchbury [27] stated that 'interpretation of many aspect of cytokinin biochemistry is dependent on a much better understanding of the relationship of their metabolism to that of other purines'. Much is known of cytokinin metabolism, but no common metabolic pattern has emerged. Several factors may have contributed to this complexity. The stage of plant development [27, 43, 119], physiological condition [75, 162], organ type [14, 27], plant species used [20], concentration of supplied compounds [213], and method of application [223] have all been shown to have an effect on the metabolism of exogenous and endogenous cytokinins.

7.1 *Inter-conversion within the active cytokinin pool*

The free base, nucleoside and mononucleotide forms of cytokinins all appear to be readily inter-convertible in plant tissues [121, 215]. These cytokinin species are considered the functional forms [126]. The early formation of the 9-riboside ([9R]BA) and 9-ribotide of BA ([9R-MP]BA) by many systems as the principal metabolites of BA [12, 72, 126, 158, 220, 223] could be a mechanism for maintaining an active cytokinin pool. This would ensure a continued supply of precursors for subsequent conversion to the base (or active form). Di- and tri-nucleotides of cytokinins do not appear to contribute significantly to this active pool [106].

Should the biosynthetic pathway for BA, like that for 6-)3-methylbut-2-enylamino)purine (iP) proceed at the nucleotide level [201] then subsequent conversion to base and riboside would be expected. Cytokinin bases can be continuously catabolised by various enzymes to form adenine and other degradation compounds [67, 134], resulting in a loss of cytokinin base. Such a loss may need to be replenished in order to maintain the levels of available cytokinin (the so called 'active form') [34]. De-glucosylation [65], de-alanylation [239], deribosylation [34], and de-phosphoribosylation [221] would provide the needed cytokinin base.

The enzyme catalysing inter-conversions within the active pool are likely not cytokinin-specific, but rather those which catalyse analogous reactions for adenine, adenosine, and AMP [121, 145]. Other workers [221] have viewed such enzymes as cytokinin-specific.

Burch and Stuchbury [27] listed a series of reactions and the enzymes responsible for their inter-conversion (Table 1). The analogous conversions of the cytokinin 6-(benzylamino)purine are also shown.

Two mechanisms for the incorporation of purines into nucleotides have been proposed:

1. A two-step process involving first *nucleoside phosphorylase* to yield a cytokinin riboside and then *adenosine kinase* to catalyse nucleotide formation.
2. A one-step process involving a direct transfer of a ribose-5^1-monophosphate group from α-5-phosphoribosyl-1-pyrophosphate (PRPP) to the base, catalysed by an *adenine phosphoribosyltransferase*.

A one-step phosphoribosylation is not universally accepted. Evidence exists to support both the one-step [47, 104] and two-step [216] pathways. The two-step route may be limited by the restricted occurrence of *nucleoside phosphorylase*, rather than by *adenosine kinase* activity, which appears ubiquitous in plants [48].

7.2 *Conversion of benzyladenine to its riboside (BA → [9R]BA)*

Ribonucleosides have been shown to be formed in plant tissues when the corresponding base was supplied [195]. The significance of *adenosine phosphorylase* activity in purine salvage reactions has been the subject of considerable debate. Phosphoribosylation catalysed in a single step by adenine phosphoribosyltransferase (APRT) is viewed as the main pathway [109]. However, in APRT-lacking mutants of *Arabidopsis*, limited formation of [9R-MP]BA revealed some activity of the two-step reaction involving adenosine phosphorylase [145].

Although detected in bacterial systems [90, 188], the occurrence of adenosine phosphorylase in plants was initially questioned [48], and is still viewed by some researchers [27] as limited in distribution. This enzyme was purified from wheat germ cells by Chen and Petschow [39]. Conversion of the base to the riboside requires the addition of ribose-1-phosphate. In the presence of inorganic phosphate, phosphorolysis of nucleosides occurs [32, 188]. However, Chen and Petschow [39] noted that the equilibrium constants for the phosphorolysis of [9R]iP and iP indicate that nucleoside formation is in the favoured reaction. Chen [32] suggested that purine nucleoside was the enzyme catalysing cytokinin nucleoside formation, as distinct from purine nucleoside phosphorylase which is generally considered to be inactive towards Ade, Ado, and cytokinin nucleosides [242]. Senesi et al. [188] were the first to clearly distinguish adenosine phosphorylase from purine nucleoside phosphorylase. Adenosine was not a substrate for purine nucleoside phosphorylase, unlike the nucleosides of hypoxanthine and guanine.

7.3 *Conversion of BA riboside to its base ([9R]BA → BA)*

Formation of the base may involve a deribosylation of the free riboside [105, 208] or may be the result of a direct (reversible) dephosphoribosylation of the nucleotide [139]. Cytokinin base has been reported as a metabolite formed from the nucleoside [159, 215], and may represent an activation step [180].

The products of the hydrolytic nucleosidase from *Lactobacillus pentosus* was shown [231] to be the purine, and free ribose. The enzyme was not expected

Table 1. Inter-conversion within the active cytokinin pool, catalysed by non-specific enzymes of adenine metabolism

Enzyme	Class	Reaction catalysed for Ade	Analogous BA conversion
5^1-nucleotidase	EC 3.1.3.5	AMP + H_2O → Ado + Pi	[9R-MP]BA + H_2O → [9R]BA + Pi
Adenosine nucleotidase	EC 3.2.2.7	Ado + H_2O → Ade + ribose	[9R]BA + H_2O — BA + ribose
Adenine phosphoribosyl transferase	EC 2.4.2.7	Ade + PRPP → AMP + PPi	BA + PRPP → [9R-MP]BA + PPi
Adenosine phosphorylase	EC 2.4.2.1	Ade + R-1-P → Ado + Pi	BA + R-1-P → [9R]BA + Pi
Adenosine kinase	EC 2.7.1.20	Ado + ATP → AMP + ADP	[9R]BA + ATP — [9R-MP]BA + ADP

Pi – inorganic phosphate; PPi – inorganic diphosphate; R-1-P – ribose-1-phosphate; PRPP – α-5-phosphoribosyl-1-pyrophosphate; Ade – Adenine; Adenosine – Adenosine.

to exist because of the wide distribution of the phosphorolytic nucleosidase in animal tissues and in micro-organisms. Whitty and Hall [234] termed this enzyme isolated by Wang [231] 'purine nucleoside hydrolase'.

Adenosine nucleosidase catalyses the irreversible deribosylation of Ado, to give Ade and ribose [26]. Three separate adenosine nucleosidase enzymes were partially purified from tomato roots and leaves [26]. These workers found the conversion of Ado to Ade to be inhibited by the presence of [9R]BA, with substantial differences in the pattern of inhibition evidenced for each of the three enzymes. Earlier, Chism et al. [40] distinguished between cytokinin nucleosidases and adenosine nucleosidases in tomato fruits. When the $\underline{N}^6$-amino group of Ado was replaced by an isopentenyl amino sidechain in substrates of adenosine nucleosidase, the K_m value of the reaction was decreased by a factor of 1.7 [34]. The cytokinin base, BA, appeared a suitable substrate in this reaction, as the adenosine nucleosidase exhibited a specificity for Ado and $\underline{N}^6$-derivatives of Ado. From wheat germ cells, a partially purified adenosine nucleosidase (EC 3.2.2.7) catalysed the irreversible hydrolysis of the riboside of iP ([9R]iP) to iP, and adenosine to adenine [32, 39]. The activity of such adenosine nucleosidases appears to depend on the plant tissue investigated [159, 203]. Significant differences in adenosine nucleosidase activity were detected between wild-type and domesticated plant species [116].

Adenosine nucleosidase activity has also been detected in soybean [142], beet [172], and barley leaves [81].

7.4 *Conversion of benzyladenine riboside to its nucleotide ([1R]BA — [9R-MP]BA)*

Enzymic preparation of mono-nucleotides from $\underline{N}^6$-substituted adenosines and ATP is catalysed by adenosine kinase (EC 2.7.1.20). Such activity has been found in both yeasts and higher plants [48]. Adenosine kinase activity was demonstrated in buds of *Cicer arietinum* and in suspension-cultured cells of *Acer pseudoplatanus*.

Time course studies with *Phaseolus vulgaris* [179] and *Dianthus caryophyllus* [216], have indicated formation of cytokinin nucleotides from the corresponding nucleoside. More direct evidence was provided by Chen and Eckert [33] who reported that cytokinin nucleoside could be converted to the nucleotide (5^1-monophosphate) by adenosine kinase isolated from wheat germ cells. The phosphorylation of [9R]iP depended upon the presence of ATP and Mg^{2+}. The enzyme activity responsible for such synthesis was considered by Doree and Terrine [48] to be ubiquitous in plants.

7.5 *Conversion of benzyladenine nucleotide to its riboside ([9R-MP]BA → [9R]BA)*

Following application to soybean callus, [9R-MP]Z was rapidly metabolised to both the riboside and the base [221]. Ribonucleosides can be formed from the corresponding ribonucleotide or from the cytokinin base [32, 191]. Should the one-step pathway of nucleotide synthesis be dominant, then conversion of applied cytokinin base to the riboside may proceed through phosphoribosylation of the base to the nucleotide, followed by conversion to the riboside [39].

Conversion of cytokinin ribonucleotide to its nucleoside may be catalysed by 5^1-nucleotidase [35]. Such conversions have been indicated by time-course studies [130] and demonstrated during *in vitro* investigations [32]. This cytosolic enzyme consists of at least two forms, referred to as the F-1 and F-2 5′-nucleotidases, which specifically hydrolyse purine ribonucleoside-5^1-phosphates [32]. Notably, 5^1-nucleotidases have been reported [35] to show

almost equal affinity toward the mono-, di- and tri-phosphates of adenosine (AMP, ADP and ATP). The extent of dephosphorylation of cytokinin ribonucleotide in plant cells by acid phosphatase and membrane-bound 5[1]-nucleotidase remains to be investigated [35].

7.6 *Conversion of benzyladenine to its nucleotide (BA → [9R-MP]BA)*

Quick metabolism of base to nucleotide has been demonstrated for both lower [63] and higher plants [79, 139]. A one-step purine salvage reaction catalysed by adenine phosphoribosyltransferase (APRT) is seen as the predominant pathway in plants [139, 145], as this enzyme activity is high enough to account for the salvage of Ade into AMP. Further, some researchers [109] maintain, despite the work of Chen and Petschow [39], that the presence of adenosine phosphorylase in plants has not been unequivocally demonstrated. From a time-course study on *Acer pseudoplatanus* cell culture, Doree and Guern [47] provided evidence to show that the synthesis of $\underline{N}^6$-substituted nucleotides does not proceed through a two-step reaction, but rather through the direct transfer of ribose-5[1]-monophosphate. 6-(Benzylamino)purine was a suitable substrate.

Extracts from soybean (cv. Acme) similarly yielded APRT activity, as did senescing barley leaves [157]. This enzyme from soybean was inhibited by AMP (product feedback inhibition), and stimulated by ATP. The monophosphate of BA ([9R-MP]BA) was also found to inhibit AMP production, though not to the same extent (13 vs. 92%). Soybean callus contacting kinetin in the agar medium was shown to have increased APRT activity [157].

Adenine phosphoribosyltransferase has been partially purified from tobacco pith tissue cultures [33], and from wheat cells [32]. This enzyme was also extracted and partially purified from Jerusalem artichoke shoots [111, 112]. Phosphate ions and thiol-reducing substances were required to stabilise it.

However, Chen [32] showed that in wheat germ cells, cytokinin nucleotide is not preferentially formed by this one-step pathway as iP has a high K_m value. Krentisky et al. [101] had earlier reported that the enzyme binds to adenine through the 6-amino group and the 3- and 7-nitrogens. Chen [32] was thus not surprised to find iP (an adenine analogue with a modified 6-amino group) to show reduced ability as a substrate. It was suggested that a different form of this enzyme may exist for cytokinin bases in wheat. A later investigation revealed that APRT from the cytosol of wheat germ was capable of phosphoribosylating BA [38]. However, the ratio of $V{:}K_m$ indicated that adenine is approximately two-fold more efficient than BA as a substrate. Lee and Moffatt [109] have more recently purified and characterised an APRT from *Arabidopsis thaliana* which catalysed phosphoribosylation of BA. However, it was again not possible to fully resolve the physiological role of APRT with respect to BA.

In summary, the base, riboside and nucleotides of cytokinins appear to be readily inter-convertible within plant tissues. Enzymes responsible for catalysing these reactions are not likely to be cytokinin-specific, although in some tissues specific enzymes may be present [40]. The extent to which one-step or two-step phosphoribosylation of cytokinin bases occurs appears to be a function of the plant system investigated.

8. Benzyladenine glucosides

The metabolites of BA include a group of N-glucosides in which the sugar moiety is linked to a purine ring nitrogen atom. These are [3G]BA, [7G]BA and [9A]BA, of which the 3- and 7-glucosides are considered to be particularly unusual [12]. Transglycosylation reactions in which glucose is transferred enzymically from a glucoside to the free base BA to yield a different glucoside have not yet been reported [119].

Cytokinin O-glucosides, where glucose is substituted in the $\underline{N}^6$-sidechain of a molecule such as (2OH) BA, have not been observed for BA, as they have for zeatin [91]. The O-glucosidic linkage in such compounds as (OG)Z and (OG)DHZ confers a greater liability to acid and β-glucosidase hydrolysis [166, 212, 219, 225] than has been observed with any N-glucosidic bonds. As a result, O-glucosides probably serve as translocatory [210, 217] and storage forms [75, 211], unlike the more stable N-glucosides. As the presumed roles of these two glucoside types fundamentally differ, analogies which may be made are limited. Accordingly, cytokinin-O-glucosides are not currently reviewed. However, it is noteworthy that not only glucose, but xylose has also been identified (from *Phaseolus vulgaris* embryos) as a cytokinin conjugate ((OX)Z) [147, 149]. Turner et al. [205] isolated and partially characterised the enzyme (UDP xylose: zeatin-xylosyl transferase) catalysing such conjugation, and showed its specific requirements for UDP-

xylose. To date, cytokinin-O-xylosides have only been detected in members of the family Leguminosae. Should the natural occurrence of an O-glucoside of (2OH)BA or (2OH)[9R]BA be indicated, then the existence of analogous O-xyloside of BA in selected plant species is conceivable. In this regard, the finding of (2OH)[9R]BA in *Populus* × *robusta* [94] and the recent identification of (2OH)BA as a natural cytokinin [197] makes the hypothetical existence of (OG)BA and (OX)BA more feasible.

Both [7G]BA and [9G]BA have been confirmed by synthesis [41], to be β-D-glucopyranosides. Parker et al. [175] considered the identification of the glucosides of zeatin and BA to be the first unequivocal evidence for the occurrence of purine glucosides in living tissues. Letham et al. [124] later demonstrated that glucosylation of such purines is not restricted to only $\underline{N}^6$-substituted adenines with strong cytokinin activity.

Cytokinin N-glucosides have not been detected in xylem sap, and hence are apparently not supplied to the leaf from the root [50]. These N-glucosides are much less active in bioassays than the O-glucoside or the parent molecule [88, 118, 220]. They have alternatively been described as having 'enhanced metabolic stability' [96, 126, 173]. If these metabolites are the functional form of BA, then they would probably exhibit high cytokinin activity. The stability of N-glucoside metabolites is possibly due to their resistance to degradative enzymes [127] or to their compartmentation [126].

Letham et al. [126] found that formation of the 3-, 7- and 9- glucosides of BA was not dependent on BA concentration, in which case formation of the metabolites may not simply be a mechanism for inactivating physiological excesses of BA. Similarly, the rate of BA glycosylation in radish cotyledons [126] and tobacco cells [77] was found to be relatively insensitive to large differences in the concentration of supplied BA [165].

Entsch and Letham [58] claimed that the physiological significance of the 7- and 9-glucosides of cytokinins is uncertain, although it has been suggested [170] that they are storage forms of the hormone rather than the product of a detoxification pathway [77]. Entsch et al. [62] proposed that cytokinin glucosides may simply be waste-products formed by glucose transferases, which catalyse the formation of glucoside metabolites characteristic of a particular species. In summary, the significance of cytokinin metabolite formation, in particular glucosylation, has been variously associated with a detoxification mechanism, a method of storage, and a mechanism for lowering endogenous cytokinin levels [193].

8.1 *The 3-glucoside of bezyladenine – [3G]BA*

When supplied to de-rooted radish seedlings, BA was principally converted to 7-, and 9-glucosides. A third minor metabolite exhibited cytokinin-like activity markedly greater than that of these glucosides [235]. It was identified [127] as the first compound with a glycosidic linkage at position 3 of a purine ring to be isolated from a plant tissue. This compound was 6-benzylamino-3-β-D-glucopyranosylpurine ([3G]BA).

The 3-glucosides have not been isolated as endogenous cytokinins from any source, although [3G]DHZ appeared as a minor metabolite when DHZ was exogenously applied to de-rooted radish seedlings [136].

Letham et al. [122] considered a number of cytokinin bioassays and compared the activities of 3-, 7- and 9-glucosides of BA. Cytokinin activity was markedly reduced by 7- and 9-glucosylation in nearly all bioassays, but 3-glucosylation of BA had little effect on activity. The 3-glucoside of BA, produced as a minor metabolite of BA in *Dianthus caryophyllus* flowers, showed higher senescence-delaying activity than either the 7- or 9-glucosides [216]. Since 3-alkyl derivatives of BA are essentially inactive [192], the high activity of [3G]BA in diverse bioassays [122, 174, 220] is probably due to cleavage of the 3-glucoside moiety to release free BA. Such cleavage has been demonstrated in radish cotyledons [119, 126] and soybean callus [220]. The 3-glucoside of BA supplied to cytokinin-dependent soybean callus was rapidly metabolised to mainly BA, and another unidentified bioactive compound [220]. Release of appreciable amounts of BA from [3G]BA was considered by Letham and Gollnow [119] to account for the high activity of this glucoside in cytokinin bioassays. The 3-glucoside has been shown susceptible to hydrolysis by almond β-glucosidase [127, 174, 220]. The 3-glucoside is hydrolysed slowly by this enzyme whereas the 7- and 9-glycosyl metabolites of BA are not hydrolysed at a detectable rate by either α- or β-glucosidase [174]. Of the three N-glucosides of BA, [3G]BA was the most readily hydrolysed by acid [220]. These authors proposed that if steric factors cannot adequately explain the greater lability of the N-C glucosidic bond of [3G]BA in the presence of β-glucosidase, then compartmentation of the various glucosides may differ. The enzyme(s) responsible for [3G]BA formation have not yet been characterised.

Although more active than [7G]BA and [9G]BA as a cytokinin, [3G]BA is still only weakly active relative to the corresponding base when applied at physiological levels in bioassays [220].

8.2 *The 7-glucoside of benzyladenine – [7G]BA*

Raphanatin ([7G]Z) was the first purine glucoside to be identified [170]. It was earlier isolated from radish cotyledons by Parker et al. [172] who reported on its activity in the radish cotyledon bioassay. Prior to the report by Parker and Letham [170], Deleuze et al. [46] had isolated the 7-glucoside of BA from sliced potato tubers and based on spectral evidence, proposed a glucofuranosyl structure. The glucose ring size and stereochemistry of the sugar linkage of [7G]BA was later investigated [51] and found to be a 7-β-glucopyranoside. Similarly, although Fox et al. [71] reported on the existence of the 7-glucofuranoside of BA in potato tuber tissue, this was later shown [120] to be the pyranoside. The 7-glucoside of BA ([7G]BA) has been shown to be the major metabolite in tobacco, another solanaceous species [77, 207].

The base appears to be the precursor for 7-glucosylation, as expected from the consideration of the 7- and 9-tautomeric positions of the purine ring, although kinetic studies indicated that the ribonucleotide is the immediate precursor [71].

The roles of cytokinin-7-glucosides in controlling hormone activity remain unclear. the 7-glucosides of cytokinins are metabolically stable [77, 170], and weakly active relative to the unsubstituted cytokinin [103, 235]. McGaw and Horgan [138] considered [7G]BA as a deactivation or detoxified cytokinin form, which was biologically inactive. Laloue et al. [105] considered 7-glucosylation of BA as a (terminal) inactivation step as its formation was 'practically irreversible' and the rate of reutilization extremely slow [77]. When the amount of [7G]BA present in tobacco cells did not increase proportionately following further addition of BA, Gawer et al. [77] reasoned that 7-glucosylation formation may not be a detoxification step.

In contrast to the proposed terminal inactivity of [7G]BA, several researchers have viewed the 7-glucosides as storage forms of cytokinins [103, 104, 119, 170]. Laloue [103] suggested that they are storage forms as they are stable with respect to degradation that occurs upon N^6-sidechain removal [105] and because they can be converted to cytokinin nucleotides [77]. These authors suggested that this conversion is indirect, via the transient formation of BA. As β-glucosidases do not substantially. hydrolyse [7G]BA, then the existence of an enzyme which removes the glycosyl moiety at position 7 of the purine ring and simultaneously attaches a phosphoribosyl group should be considered.

Letham and Gollnow [119] suggested that the cytokinin-7-glucoside of zeatin may be a translocation and a storage form, given its resistance to degradation, its production at sub-optimal levels (hence not a detoxification form), and movement in radish seedlings [126].

Laloue [103] considered the effect of [7G]BA on cell division in suspension cultures of *Nicotiana tabacum*. He found that cytokinin-7-glucosylation was not involved in the expression of the biological activity of cytokinins. This report conflicted with the view of Fox et al. [71] who considered cytokinin-7-glucosides to be the 'active forms', as this metabolite was the only cytokinin species containing the intact cytokinin moiety that remained in actively growing cytokinin-requiring tobacco tissue in the long term. These authors had reasoned that as [7G]BA was not degraded through sidechain cleavage, the 7-glucoside may thus be the active form of the cytokinin. Additionally, substantial growth was detected in the soybean callus bioassay.

Soybean callus degraded [7G]Z to [7G]Adenine [168], showing that oxidation of [7G]BA may in fact occur. McGaw and Horgan [138] found that [7G]Z was metabolised to adenine, adenosine and [7G]adenine within two days. By inference, one would expect [7G]BA to be susceptible to β-glucosidase, although this has not yet been demonstrated. Van Staden and Drewes [220] later showed very little degradation by this enzyme, much less than for the more labile [3G]BA.

The lack of agreement on the biological significance of cytokinin-7-glucosides highlights the confusion surrounding most cytokinin metabolite roles. A lack of uniformity with respect to systems investigated and particularly bioassays employed, has probably been a major cause for such apparent contradictions. However, when one considers the wide array of bio-responses elicited by cytokinins, the varied experimental approaches are placed in context.

In summary, although early evidence permitted the interpretation of [7G]BA as an active or storage form of cytokinin, this compound is currently widely viewed as an inactive product of deactivation or detoxification mechanisms. This glucoside may be degraded further to oxidation products.

8.3 *The 9-glucoside of benzyladenine – [9G]BA*

Letham et al. [120] demonstrated the production of [9G]BA by potato tuber tissue. A considerable amount of this compound was found by Zhang et al. [241] to be produced in soybean leaves. The 9-glucoside of BA is a stable metabolite in radish cotyledons [235]. This glucoside was detected by Van Staden et al. [216] as a major metabolite in cut carnation flowers. Cytokinin base (BA) supplied to the stems was later recovered partly as [9G]BA in the stem, petals and receptacle.

The partially characterised cytokinin extract from rice roots and presumed to be a 9-glucoside [237] is more likely O-glucosylzeatin [91, 198, 230]. Following this report, Letham [117] identified a 9-glycoside of zeatin from corn kernels, although the identity of the sugar moiety was not established. Subsequently, the 9-glucoside of zeatin was isolated as a major metabolite from roots to *Zea mays* [171].

Oil palm *Elaeis guineensis* callus supplied with kinetin produced [9G]KIN as the major metabolite [97]. As oil palm cultures do not require added cytokinins, these authors suggested inactivation of an excessive cytokinin load had occurred. Jones and Hanke [97] noted that in cytokinin-autonomous *Elaeis* cultures, added cytokinins have not been shown to improve callus growth. Cytokinin-9-glucosides may then be formed in an inactivation or detoxification pathway. Earlier reports [170] have considered [9G]BA to be a storage form.

The stability of [9G]BA has been demonstrated: [9G]BA is resistant to enzymic degradation by *Escherichia coli* nucleoside phosphorylase and a nucleoside hydrolase from *Cicer arietinum*, unlike [9R]BA which was susceptible [80]. Van Staden and Drewes [220] similarly found [9G]BA to be a stable, inactive metabolite in soybean callus. When Guern [80] and co-workers were experimenting with [9G]BA, no purine glucosides were known to occur naturally. Recently, [9G]BA has been identified as a naturally occurring cytokinin [155].

When [9G]BA was injected into *Cicer arietinum* seedlings, it was apparently readily translocated without appreciable enzymic modification [80]. However, cytokinin-9-glucosides have not been detected as endogenous translocatory forms.

As [9G]BA is weakly active relative to BA [122, 219, 220, 235] specific inhibitors of the glucosylating enzymes may constitute a mechanism for elevating endogenous cytokinin levels. Although the N-glucosides (7- and 9- particularly) are generally inactive forms of cytokinins [126], [9G]BA has been found similarly active to free BA in retarding the senescence of radish leaf discs [122].

In summary, the generally low activity of [9G]BA in a variety of cytokinin bioassays indicates that this compound is probably an inactivation or detoxification form.

8.4 *The glucose-ribose conjugate – [9R-G]BA*

A new hexose (probably glucose) conjugate of [9R]Z, susceptible to β-glucosidase cleavage, was detected in Douglas-fir (*Pseudotsuga menziesii*) [152]. Immunoaffinity and mass spectral techniques indicated that this compound is not (OG)[9R]Z. It was suggested that the hexose moiety is attached to the purine ring or to the ribose group. The latter position was favoured owing to the ease of hydrolysis by β-glucosidase. However, N-conjugation does not totally preclude hydrolysis by β-glucosidase [221]. Earlier, Taylor et al. [202] had detected a novel ribosyl zeatin glycoside which could be the same compound further characterised by Morris et al. [152], but in a different coniferous species (*Pinus radiata*). These workers suggested that the hexose moiety was glucose. Van Staden and Mallett [223] and later Van Staden and Bayley [215] detected a glucosylated form of [9G]BA following BA application to tomato shoots. Further structural characterisation of this unknown metabolite was not attempted.

A disaccharide of BA, 6-(benzylamino)-9-(glucosylribosyl)purine was identified by Blakesley et al. [21] as the major metabolite in *Gerbera jamesonii* callus. The exact position of the ribose-glucose linkage was not determined. Unaware of the report on *Gerbera*, Auer and Cohen [9] reported on [9R-G]BA formation in *Petunia* leaves, and proposed a linkage of the ribose at the 3-position to glucose at the terminal (1) position. Morris et al. [152] suggested a possible storage role for this metabolite. In citing the observed activity of [9R-G]Z in the soybean hypocotyl bioassay [201]. Auer and Cohen [9] suggested that [9R-G]BA may contribute to the pool of inter-convertible active cytokinins. As further circumstantial evidence, these authors noted that [9R-G]BA formation was associated with increased shoot organogenesis in *Petunia* explants.

The production of glucosylated ribosides of cytokinins by plants of such diverse taxonomic relation as the Pinaceae, Compositae and Solanaceae, indicates that these compounds may be commonly and widely produced by plants, albeit as minor metabolites.

8.5 *The benzyladenine – alanine conjugates [9Ala]BA*

Lupinic acid ([9Ala]Z), the alanine conjugate of zeatin, was first identified in *Lupinus angustifolia* seedlings [129], and later shown [173] to exhibit only very weak activity in the radish-cotyledon cytokinin bioassay. Lupinic acid was at that time 'the only plant product known in which an amino acid moiety is conjugated to a nitrogen atom of the purine ring'.

A fraction 'C', isolated by Dyson et al. [54] from soybean callus, was shown to be a stable, long-lived BA derivative with cytokinin activity. This compound was converted from 20–25% of the applied BA and was metabolically related to [9R-MP]BA. This metabolite was considered by Deleuze et al. [46] to be 6-benzylamino-7-glucofuranosylpurine. Later researchers [56] have questioned this earlier assumption, believing the compound to be the alanine conjugate of BA.

Letham et al. [125] and Elliott and Thompson [56] reported on the existence of [9Ala]BA in *Phaseolus* seedlings and soybean callus respectively. This conjugate was found as the principal metabolite of BA in soybean [24]. These authors considered [9Ala]BA to be an inactivated form of BA. As [9Ala]BA showed only slight senescence retarding activity, Letham et al. [122] came to a similar conclusion. The use of inhibitors of alanine conjugation [238] has provided further circumstantial evidence to support this notion. Zhang et al. [241] noted that derivatives of BA with a slowly cleaved substituent at N-9 (to yield free BA), could be more effective than BA in exerting cytokinin activity. Such a case may be when only one or a few cytokinin applications are practicable for evoking a response. In contrast to these observed activities, [9Ala]BA at high concentrations was almost as active as BA in the *Amaranthus* bioassay [122].

As BA is probably not a substrate for cytokinin oxidase in all plant systems alternative inactivation through N-conjugation may account for the accumulation of [9Ala]BA in soybean callus [122]. Zhang et al. [241] investigated the suppression of [9Ala]BA formation in order to enhance the senescence retarding activity of BA. Inhibition of [9Ala]BA formation was accompanied by a greater degree of $\underline{N}^6$-benzyl cleavage, associated with the production of adenine and adenosine. Consequently, these authors suggested that $\underline{N}^6$-sidechain cleavage and alanine conjugation are alternative mechanisms for BA inactivation in soybean leaves. The low cytokinin activity of the alanine conjugates is probably due to an inability of tissues to readily cleave the alanine moiety and release free cytokinin [122]. The alanine conjugate of BA was largely unmetabolised in soybean leaf discs; no BA formation was detected [239]. However, zeatin was shown to be released from [9Ala]Z, which would explain the observed minor activity of [9Ala]Z in the soybean bioassay [168]. This raises the possibility that alanine conjugates are a further storage form of cytokinin. Palni et al. [168] viewed the observed stability of the alanine conjugate of zeatin to result from its compartmentation, and therefore protection against the enzymes involved in sidechain cleavage.

In soybean, rapid metabolism of BA to [9Ala]BA was confined to the first 24 hours after application, and was associated with uptake of the supplied BA. Letham et al. [122] hypothesised that remaining BA may be sub-compartmented in the cell, separated from the action of inactivating enzymes. Such is the extent of alanine conjugation with BA in soybean leaves that BA analogues have been found more effective than BA in regarding soybean leaf senescence [240].

The question was posed by Letham et al. [125] as to whether alanine conjugates of cytokinins are confined to the Leguminosae. This BA conjugate has been identified from *Phaseolus vulgaris* [125], *Glycine max* [56], *Lupinus angustifolius* [129], and probably *Lupinus luteus* [71].

9. Enzymes specific for N-conjugation of cytokinins

With the exception of cytokinin oxidase [234] research into enzymes of cytokinin metabolism had not been reported until 1979. Entsch et al. [62] then reported on the preparation of one of two isozymes from radish cotyledons which glycosylated cytokinins.

Letham and Palni [121] cited three characterised and purified enzymes known to show specificity for cytokinins, namely *cytokinin oxidase*, *cytokinin-7-glucosyl transferase*, and *β-(9-cytokinin)-alanine synthase*. Since this review was published, several other cytokinin-specific enzymes have been identified. These include *zeatin reductase* [131] and a *cis-trans*-isomerase of zeatin [13]. The existence of a 'cytokinin-β-glycosidase' has also been reported, as has a *cytokinin O-xylosyltransferase* [205].

The metabolism of exogenously applied cytokinins, including BA [21, 220] indicates the presence of other, as yet uncharacterised enzyme systems.

9.1 *Cytokinin-7-glucosyl transferase*

Radish cotyledon extracts yielded a single enzyme system collectively known as cytokinin-7-glucosyl transferase comprised of two enzymes/isozymes. These converted BA into 7- and 9-glucosides when uridine diphosphate glucose (UDPG) was supplied as a glucose donor [58, 60]. Cytokinin-7-glucosyl transferase produced the two glucosides in different proportions; the major isozyme favoured production of the 7-glucoside, and the minor glucosyl transferase formed the 7- and 9-cytokinin glucosides in similar proportions [62]. Entsch and Letham [58] expressed surprise to find that the 7- and 9-glucosides were not formed by separate enzymes, especially considering the small size of the enzyme (46,000 daltons).

However, in view of the many systems in which cytokinin-9-glucosides are produced as the major metabolite [19, 97, 145], it is possible that a separate cytokinin-9-glucosyltransferase exists, or a 'cytokinin-7-glycosyltransferase' which forms both the 7- and 9-glucosides, yet favours production of the latter. As with cytokinin oxidase-type systems [31, 100], different enzymes or isozymes of cytokinin-7-glucosyltransferase with a similar function are likely to occur in a range of plant tissues.

Although a trace enzyme, the glucosyl transferase studied by Entsch et al. [62] could exert a regulatory role in metabolism since cytokinins occur in trace amounts, evoking key responses at the sub-nanomolar level.

Entsch et al. [62] considered that inhibitors of cytokinin-7-glucosyl transferase merit study, as 'a stable, effective, and specific inhibitor *in vitro* could be a valuable physiological tool and a means of elevating endogenous free cytokinin levels by suppressing formation of the very weakly active 7-glucosides. Several studies have been undertaken in this regard [55, 89, 169, 200]. Greater effort in this area could result in the development of regulatory mechanisms optimizing tissue culture systems.

9.2 *β-Glucosidase*

Hydrolysis of O-glucosides of zeatin-like cytokinins may function in controlling cytokinin activity. In this regard β-glucosidases would play an important role [212]. Until recently, non-specific β-glucosidases were considered to be involved in cytokinin metabolism. However, the existence of a specific 'cytokinin-β-glucosidase' has recently been reported. Estruch et al. [65] transformed tobacco tissues with a *rol* C oncogene from the T-DNA of *Agrobacterium rhizogenes*. This gene coded for a 'cytokinin-β-glucosidase' which was capable of hydrolysing [9G]BA to its free base. Despite this report, Kaminek [99] considered such hydrolases to be either absent or inactive in normal plant cells.

Almond β-glucosidase did not hydrolyse cytokinin 7- or 9-glucosides *in vitro* [50], although limited cleavage of [7G]Z has been reported in radish tissues [126]. The resistance to hydrolysis was presumed due to their C-N glycosidic linkages [91] although enzymic degradation of a similar bond in [3G]BA [220] remains unaccountable.

β-Glucosidases have functions unrelated to growth [95], so their activity in regard to cytokinin metabolism is not surprising. Hughes [95] found two distinct β-glucosidases produced by clover callus. It was shown that β-glucosidase activity and concentration varied both between plants and between organs of the same plant. Genetic variation and the environment were cited as causal factors of this. McCreight et al. [135] later found different forms of β-glucosidase in different plantorgans of the same species.

9.3 *β-(9-Cytokinin)-alanine synthase*

The enzyme which converts cytokinin base to considerably less active alanine conjugates is known as lupinic acid synthase or β-(9-cytokinin)-alanine synthase [241]. This enzyme is classed as C-N-ligase lupinic acid ([9Ala]Z) [59, 154]. Enzyme-catalysed formation of lupinic acid was determined by ^{14}C incorporation from O-acetyl-srine-3-^{14}C as a substrate into lupinic acid. Murakoshi et al. [154] demonstrated that enzymes from different plant species which catalysed the synthesis of β-substituted alanines from O-acetyl-L-serine had different specificities; not all enzymes recognised zeatin as a substrate.

β-(9-Cytokinin)-alanine synthase was isolated from developing lupin seeds by Entsch et al. [61]. In this report, a number of adenine derivatives were shown to serve as substrates, although preference was shown for compounds with high cytokinin activity, including BA and Kinetin. In the reverse direction, a small amount of base was formed [168]. Although indole auxins have a similar type of ring structure to purines, IAA was not a substrate for lupinic acid synthase [61].

10. Oxidative catabolism of benzyladenine

10.1 *Cytokinin sidechain cleavage*

Cytokinin activity is conferred on (intact) purine molecules through the possession of a suitably-structured N^6-sidechain [132, 226]. When this sidechain is (oxidatively) removed, relatively inactive degradation products are formed. Further loss of activity occurs on disruption of the adenine moiety [18, 191]. The cytokinin-specific enzyme responsible for such sidechain cleavage is cytokinin oxidase, an enzyme common to both lower [4] and higher [234] plants.

The activity associated with oxidative catabolism was detected in many early cytokinin metabolic studies, involving the degradation of cytokinin bases and ribosides of both naturally-occurring [37, 144, 161] and synthetic cytokinins [57, 68, 69, 72, 134]. Fox et al. [72] even found a 'benzoic acid-like compound' to be a product of the cleavage of BA. A re-investigation revealed this 'benzoic acid-like compound' to be the aldehyde sidechain cleavage product [24].

Since the initial isolation and characterisation of cytokinin oxidase from *Zea mays* [234], there has been general agreement that the presence of a double bond in the isopentenyl sidechain of a cytokinin renders it susceptible to oxidation. A great number of studies have reported on such oxidation [79, 96, 165, 168, 170, 214].

The converse view that cytokinins with saturated aliphatic (DHZ) or ring (kinetin or BA) sidechains do not serve as substrates for cytokinin oxidase, has also been widely advocated [86, 121, 126, 137, 160, 167, 234]. Despite regular reports on degradation product formation, cytokinin oxidase-mediated catabolism of both BA and kinetin remains controversial. This scepticism is well substantiated, for to date, no cytokinin oxidase preparations isolated from plants have appreciably utilised BA [29, 100]. Despite the lack of activity observed *in vitro*, a large body of circumstantial evidence has accumulated which is indicative of *in vivo* BA degradation. Many [8-^{14}C]-labelled products of oxidative catabolism (adenosine, adenine, adenine nucleotides, ureides) have been identified following application of [8-^{14}C]BA to a wide variety of plant systems [17, 54, 67, 72, 104, 134, 203, 216].

The degradation of BA and kinetin in many systems has been attributed to a separate enzyme system [121], distinct from cytokinin oxidase. These authors attributed the cleavage of furfuryl groups from the N^6-position (to yield adenine and its derivatives), to such a system. An alternative theory for observed loss of the benzyl group was proposed by McCalla et al. [134]. They considered that C-8 from [8-^{14}C]BA could be lost to the 'one carbon' pool with subsequent reincorporation into newly synthesised purine. Some 12 years prior to Whitty and Hall's report of cytokinin oxidase [234], McCalla et al. [134] also considered the possibility of direct enzymic removal of the benzyl group.

Zhang and Letham [239] hypothesised that N^6 de-benzylation of BA 'probably involves an imino intermediate formed enzymically by elimination of a hydrogen atom from both the NH group at position 6 and the benzylic methylene'. In species where cytokinin oxidase catabolise BA [67, 239], it is possible that such activity can only be expressed if the enzyme facilitating production of the imino form is also present and active. The imino-purine intermediate postulated by Whitty and Hall [234] has been isolated [31] as an intermediate in the degradation of isopentenylated cytokinins. As BA has not been shown to be a substrate for cytokinin oxidases in any *in vitro* assays to date [100], the importance of an enzyme catalysing formation of an imino intermediate should be accorded more consideration.

Chatfield and Armstrong [31] provided an indication that distinct isozymes of cytokinin oxidase may exist. Cytokinin oxidase from *Vinca rosea* crown gall tissues [137] appeared to be a different system to that partially purified from maize kernels [234]. The molecular weights of the two enzymes, determined by gel filtration, are very different: 944400 ($\pm$ 10%) for maize and 25100 ($\pm$ 10%) for *Vinca rosea*. The two enzymes exhibited similar substrate specificities; neither recognised BA as a substrate. Thus, evidence for heterogeneity in cytokinin oxidase activity is provided by the range of molecular weight estimates for the enzyme from different plant tissues. Chatfield and Armstrong [31] suggested that this heterogeneity is related to glycosylation and noted that should this be confirmed, then new implications for the compartmentation and regulation of this enzyme would arise. A clear implication of such heterogeneity is that specific cytokinin oxidases may exist which preferentially attack BA, rather than isopentenylated cytokinins. In all studies to date, *in vitro* assays have been performed with enzymes extracted from tissues in which low *in vivo* degradation has been observed. Enzyme extracts from tissues which are known to substantially degrade exogenously

applied BA e.g. *Glycine max* cv. Acme) [67] could well prove rewarding in this regard.

Cytokinin oxidase is able to utilise a number of different cytokinin substrates, including bases, ribosides [190], N-glycosides and N-alanyl conjugates [137]. However, ribonucleotide [110, 137] and O-glucoside [186, 225] forms are thought to be resistant to cytokinin oxidase. Data has been presented which suggests that substrate induction occurs, where cytokinin degradation is promoted by cytokinins themselves [31, 153, 203]. The exact metabolic level of sidechain removal from BA is not known [105]. Several studies have shown that N-conjugates are susceptible to further inactivation through oxidation. Cytokinin oxidase is considered to be the active catalyst. Soybean callus degraded [7G]Z, [9G]Z, and [9Ala]Z to [7G]Ade, [9G]Ade and [9Ala]Ade respectively [168]. These compounds appeared as minor metabolites. Prior to this report, Letham et al. [126] had identified [7G]Ade in radish seedlings. The 7-glucoside of zeatin was metabolised to adenine, adenosine and [7G]Ade within two days [138]. Should a cytokinin oxidase-type system fully utilise BA metabolites, one might expect these same products to be produced.

A further metabolite of BA may be adenosine-5^{1}-phosphate (AMP), which has previously been identified during metabolic studies with zeatin [52] and [7G]Z [138]. Early BA metabolic studies [47, 134] reported on the identification of adenylic nucleotides as products. However, in recent years, no reports of AMP formation from BA have been published.

McGaw and Horgan [137] considered cytokinin oxidase as a candidate for the control of endogenous cytokinin species and levels. However, Whitty and Hall [234] had earlier cautioned against assuming that the whole purpose of cytokinin oxidase is to help maintain some specific level of cytokinins. Rather, these authors viewed the rate of turnover of cytokinins to be a means of conveying information necessary for control of cellular growth. An actual role for cytokinin oxidase in the control of the endogenous levels of cytokinins is difficult to assign, when one considers certain anomalies. In *Vinca* crown gall tissue and corn kernels, the most abundant cytokinins are [9R]Z and zeatin [185]. Both these hormones are readily metabolised by cytokinin oxidase [234], the presence of which has been demonstrated in these tissues. Compartmentation of substrates probably prevents these cytokinins coming in contact with the oxidative enzyme system [137]. Evidence for such distinct compartmentation of different isozymes of cytokinin oxidase has recently been presented [100].

Plant cytochrome P-450 found in the microsomal fraction of cauliflowers was shown by Chen and Leisner [36] to exhibit oxidative dealkylation activity, much as cytokinin oxidase does. After two hours incubation of [9R]iP with cytochrome P-450, about 15–25% adenosine was formed.

If therefore appears that benzyladenine is the substrate for a cytokinin-oxidase type system which degrades this cytokinin adenine. Cytokinin oxidase may not directly utilise BA, but only following conversion to an imino intermediate. The potentially restricted distribution of an (uncharacterised) enzyme catalysing formation of this intermediate, would account for the limited degradation of BA (relative to zeatin or iP) which has been observed in many tissues.

11. Conclusion

Cytokinin metabolites possess functional, though somewhat obscure roles in plants [121, 229], contributing to either an active or inactive pool. Inactivation of cytokinin occurs through sidechain cleavage or alternatively N-conjugation, which proceeds through 9-alanylation or 7- and/or 9-glucosylation [99, 138]. The 3-glucoside is more biologically active than other cytokinin N-glucosides [122, 127] and appears reversibly sequestered [119, 220], suggesting a storage role. Internal levels of free, non-metabolised base appear important in the initiation of physiological responses [207]. Nucleosides and nucleotides are also considered as active forms [109, 121], given their ready conversion to cytokinin bases and/or interconversion [215, 221].

From a physiological viewpoint, cytokinin metabolism may be classified [93] under three headings:

1. Irreversible loss of biological activity through oxidative degradation of the $\underline{N}^{6}$ sidechain (products here referred to as 'oxidation products').
2. Irreversible conjugation with alanine or glucose with loss of, or reduction in activity (products here referred to as 'N-conjugates').
3. Reversible conjugation to (inter-convertible) compounds which are themselves active, or serve as storage forms which may be converted to active cytokinins (her referred to as the 'active pool').

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386. FLUORESCENT CYTOKININ ANALOGS

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In order to develop fluorescent cytokinin analogs with high activity, 6-alkynyl-1-deazapurine and its riboside were synthesized. Palladium-catalyzed coupling of the corresponding 6-iodide triacetate with phenylacetylene, followed by ammonolysis, provided 6-phenylethynylpurine riboside. Acid hydrolysis of the riboside gave its free base in a good yield. The 1-deazapurine bases exhibited a marked increase in fluorescence and a red shift of the emission maximum in an aqueous alkaline solution. In an *Amaranthus* bioassay, it promoted betacyanin biosynthesis more significantly than the purine counterpart, the activity of the former being close to that of N^6-benzyladenine (BA). A tobacco callus bioassay also revealed that the 1-deazapurine base was almost as strong as BA. However, the new base was less effective than expected. It may be a useful tool for studying cytokinin transport.

387. IDENTIFICATION OF NEW AROMATIC CYTOKININS IN PLANTS

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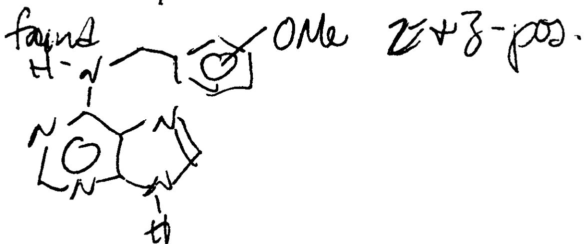
6-benzylaminopurine (BAP) and its derivatives were found as native cytokinins in various plant species, namely *Populus* × *robusta* leaves. Later isolation and equivocal identification of BAP derivatives and the high biological activity of its hydroxylated analogues stimulated the search for other natural aromatic cytokinins. We report here the isolation and identification of new cytokinin groups derived from 6-(2- and 6-(3-methoxybenzyl-amino) purine – MeoT, MemT. Six-week old *A. thaliana* cv. Colombia plants growing in the soil and leaves of field grown *P.* × *robusta* were used in this investigation. Ethanol extracts of the plant tissues were purified by SPE and IEC. As a final purification step, immunoaffinity chromatography based on polyclonal antibodies was developed for the studied compounds. Specific ELISAs of the HPLC fractions were performed for the detection and quantification. The LC(+)ESI-MS method was also introduced for decisive identification of the studied compounds.



388. DEVELOPMENT OF NEW, HIGHLY ACTIVE ANTICYTOKININS

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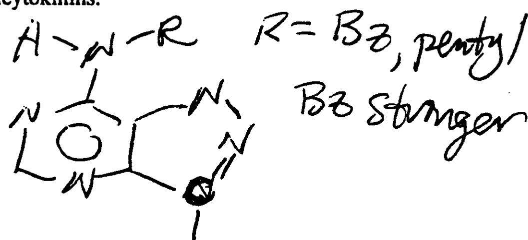
New, highly active pyrazolo[4,3-d]pyrimidines have been developed. These compounds were tested in different CDK (cyclin-dependent kinase) assays as well as anticytokinins in callus tobacco assay. Their activity was compared with the strongest anticytokinin 7-pentylamino-3-methylpyrazolo[4,3-d] pyrimidine (Hecht et al.: Phytochemistry, 12, 25, 1973) and key cytokinin-derived CDK inhibitors (olomoucine, bohemine, roscovitine). The compounds having strong CDK inhibitory activity (substituted at C7 by 2- and 3-hydroxybenzylamino, etc.) were usually week anticytokinins, but on the other hand, the cyclopentyl, cyclobutyl and n-amyl derivatives with low CDK inhibitory activity exhibited very strong anticytokinin activity. The anticytokinin activity was also tested in other bioassays (senescence, *Amaranthus*). However, the developed pyrazolo [4,3-d]pyrimidines are inactive in these bioassays as anticytokinins.



389. CYTOKININ BIND IN ATP BINDING POCKET OF CYCLIN-DEPENDENT KINASES

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Recently we have discovered an unusual specificity of cytokinin derivatives towards cyclin-dependent kinases (CDK). Subsequent X-ray structure determination of the cytokinin/CDK-2 co-crystals shed some light into the nature of cytokinin binding. Interestingly, the purine ring of adenine in these cytokinin derivatives is oriented in an unexpected and different orientation from the adenine base of the authentic substrate ATP. However, because of the different orientation of the purine ring, the contacts are formed with different atoms in the adenine. As expected, the cytokinin/CDK2 co-crystals confirm the results of the cytokinin structure-activity studies in that the N7, N3, and N9 of the purine must remain free of substitution because of the hydrogen bonds formed by these nitrogen atoms. Docking-based development of new cytokinin CDK inhibitors will be presented.

DP

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Immunodetection and Identification of N^6-(*o*-Hydroxybenzylamino)Purine as a Naturally Occurring Cytokinin in *Populus* × *canadensis* Moench cv *Robusta* Leaves[1]

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ABSTRACT

A highly specific and sensitive enzyme-linked immunosorbent assay (ELISA) was developed for 9-β-D-ribofuranosyl-N^6-(o-hydroxybenzylamino)purine [(oOH)[9R]BAP] and structurally related cytokinins. As little as 3 femtomoles of the compound could be detected by this method. Cross-reactivity studies demonstrated the specificity of four polyclonal antibodies for (oOH)[9R]BAP and its free base in preference to a range of natural cytokinins and other purines. After evaluating the method by internal standardization employing [2-^{3}H](oOH)[9R]BAP of high specific radioactivity as recovery marker by dilution analyses and by immunohistograms, it was possible to apply ELISA to quantify (oOH)[9R]BAP in plant extracts. In addition to (oOH)[9R]BAP, an unknown cytokinin reacting with the same antibody was detected in high performance liquid chromatography-fractionated extracts of mature *Populus* × *canadensis* Moench cv *Robusta*. The structure of the new compound was determined by gas chromatography-mass spectrometry and finally confirmed by synthesis as N^6-(o-hydroxybenzylamino)purine.

Most natural as well as many synthetic cytokinins contain an isoprenoid side chain attached to the N^6-position of the adenine (14). Cytokinins with an aromatic ring substituting at N^6 were supposed to occur only sporadically in a few plant species (13, 14). Horgan *et al.* (9, 10) isolated the first cytokinin of that type from fully expanded *Populus* × *Robusta* Schneid leaves and identified it as N^6-(*o*-hydroxybenzylamino)-9-β-D-ribofuranosylpurine. Later, this compound was found, together with its 2-methylthioglucofuranosyl derivative (4, 5), in the fruits of *Zantedeschia aethiopica*. Recent identification of [9R]BAP (Table I) in an old *Pimpinella anisum* L. cell culture (8) supports the idea that this compound may be the precursor of the hydroxylated aromatic cytokinins. As suggested from the biological activities, the hydroxylation of the benzyl ring of [9R]BAP in the *o*-position may represent a deactivation step, whereas the hydroxylation in the *m*-position can generate the opposite effect (11). For accurate and fast analysis of [9R]BAP and its metabolites, we developed an analytical procedure that is based on the testing of the immunoreactivity in HPLC-fractionated extracts (21, 22). Using this approach, the free base N^6-(*o*-hydroxybenzylamino)purine was identified in *Populus* × *canadensis* Moench cv *Robusta* leaves as a naturally occurring cytokinin. We also report the preparation and characterization of the ELISA for the determination of (oOH)[9R]BAP in plant extracts. Preliminary data about this ELISA have already been published (20, 21).

MATERIALS AND METHODS

Plant Material

Fully expanded leaves were collected from 1-year-old shoots of *Populus* × *canadensis* Moench cv *Robusta* in the field. Detached leaves were weighted and immediately plunged into liquid nitrogen.

Cytokinins and Other Chemicals

[9G]Z, [9R-5′P]Z, (OG)Z, (OG)[9R]Z, (diH)[9G]Z, (diHOG)[9R]Z, (diHOG)Z, and (diH)[9R-5′P]Z were purchased from Apex (Oxford, UK). (oOH)BAP was prepared by acid hydrolysis of (oOH)[9R]BAP in 1 N TFA and purified by reversed phase HPLC as described earlier (21). [2-^{3}H](oOH)[9R]BAP (0.52 TBq/mmol) was kindly supplied by Dr. J. Hanus, Institute of Nuclear Biology and Radiochemistry, Prague, Czechoslovakia. Ostsorb DEAE-cellulose, cellulose phosphate, Separon SGX C_{18} columns were from Tessek (Prague, Czechoslovakia); acetonitrile for chromatography was purchased from Merck. Triethylammonium hydrogen carbonate was from Serva; *N*-methyl-*N*-(TMS)trifluoro acetamide was purchased from Sigma. All other chemicals were from sources described elsewhere (21) or purchased from Lachema (Brno, Czechoslovakia).

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Table I. *Abbreviations Used in this Paper*

BAP, N^6-benzylaminopurine
(mOH)BAP, N^6-(*m*-hydroxybenzylamino)purine
(oOH)BAP, N^6-(*o*-hydroxybenzylamino)purine
[9R]BAP, 9-β-D-ribofuranosyl-BAP
(mOH)[9R]BAP, 9-β-D-ribofuranosyl-(mOH)BAP
(oOH)[9R]BAP, 9-β-D-ribofuranosyl-(oOH)BAP
(diH)Z, dihydrozeatin
(diH)[9G]Z, 9-β-D-glucopyranosyl-(diH)Z
(diH OG)Z, *O*-β-D-glucopyranosyl-(diH)Z
(diH)[9R]Z, dihydrozeatin riboside
(diH)[9R-5′P]Z, dihydrozeatin riboside 5′-monophosphate
(diH OG)[9R]Z, *O*-β-D-glucopyranosyl-(diH)[9R]Z
iP, N^6-(Δ^2-isopentenyl)adenine
[9R]iP, 9-β-D-ribofuranosyl-iP
K, kinetin
[9R]K, 9-β-D-ribofuranosyl-K
Z, *trans*-zeatin
(*cis*)Z, *cis*-zeatin
[9G]Z, 9-β-D-glucopyranosyl-Z
(OG)Z, *O*-β-D-glucopyranosyl-Z
[9R]Z, *trans*-zeatin riboside
[9R-5′P]Z, zeatin riboside 5′-monophosphate
(OG)[9R]Z, *O*-β-D-glucopyranosyl-[9R]Z
TMS, trimethylsilyl
TBS, Tris-buffered saline

Extraction and Purification of the Cytokinin Fraction

Cytokinins were extracted from fully expanded *P.* × *Robusta* leaves (1 g of fresh leaf material for immunodetection, 100 g for identification) after homogenization in 80% (v/v) methanol. Extracts were purified by butanol extraction followed by cation-exchange chromatography on cellulose phosphate and by combined DEAE-cellulose-reversed phase chromatography (1, 15, 21). [2-^{3}H](oOH)[9R]BAP (approximately 50,000 cpm) was used as internal standard for estimating the recovery at each purification step.

HPLC

For the immunodetection, cytokinins were fractionated on a Separon SGX C_{18} column (250 × 4 mm, particle size, 7 μm). The column was eluted with a gradient of methanol (32–56%) in 40 mM acetic acid, adjusted to pH 3.4 with distilled triethylamine (21). Fractions of 0.5 mL were collected, evaporated *in vacuo* to dryness, and dissolved in 50 μL DMSO and 950 μL TBS buffer (50 mM Tris-HCl, 10 mM NaCl, 1 mM $MgCl_2$, 0.02% [w/v] NaN_3, pH 7.5). Each fraction was analyzed by ELISA.

For GC-MS, the partially purified and filtered extracts were subjected to HPLC on a 10-μm Separon SGX C_{18} column (250 × 8 mm). Cytokinins were eluted at a flow rate of 2 mL/min using the gradient described above. The effluent was evaporated to dryness, tested by the ELISA, and fractions containing immunoreactive material were rechromatographed twice on a Hypersil ODS column (250 × 4.6 mm, 5 μm, Shandon, Cheshire, UK). The column was eluted at a flow rate of 1.2 mL/min with acetonitrile and 5 mM triethylammonium hydrogen carbonate buffer (adjusted to pH 6.5 with distilled acetic acid) using the following gradient of acetonitrile: 15% (v/v) for 10 min; 15 to 18% (v/v) over 5 min; 18 to 18.5% (v/v) over 50 min. Subsequently, the column was washed with 100% acetonitrile for 10 min and regenerated with 15% (v/v) acetonitrile in triethylammonium bicarbonate buffer. Using this procedure, cytokinin retention times were 43.8 min for (oOH)BAP and 48.88 min for (oOH)[9R]BAP. The recovery of the cytokinins was followed with [2-^{3}H](oOH)[9R]BAP. Ten-microliter aliquot volumes of each fraction from the HPLC run were investigated by scintillation counting and in duplicate by ELISA. The (oOH)[9R]BAP-immunoreactive fractions were dried using a Speed Vac Concentrator (model SVC 100H, Savant, New York). Fractions containing (oOH)[9R]BAP and its free base were dissolved in 5 μL tetrahydrofuran (20–200 μg/μL) mixed with 10 μL of *N*-methyl-*N*-(TMS)trifluoro acetamide and kept at 60°C for 1 h. Aliquot volumes of 1 μL of the supernatant were injected into the gas chromatograph.

GC-MS

A Varian gas chromatograph, model 3700 (Bremen, FRG) equipped with an injector for split-splitless injection (270°C) was directly connected to the source of the MS. The GC was furnished with a 30 m × 0.3 mm (i.d.) bonded phase (DB-1)-fused silica capillary column (J&W Scientific, Folsom, CA); the temperature program ranged from 100 to 300°C at a rate of 2°C/min, and helium (2 mL/min) was used as carrier gas. GC-MS analysis was carried out using a Finnigan MAT 312 spectrometer with an inverse Nier-Johnson geometry and a combined electron impact/chemical ionization ion source. Electron impact spectra were determined at an ionization energy of 70 eV. The GC-MS was directly coupled to a Finnigan MAT SS 300 data system.

Preparation of Cytokinin Conjugate and Enzyme Tracer

Following the procedure of Eberle *et al.* (6), (oOH)[9R]BAP was coupled via periodate-oxidized *vic*-hydroxy groups of the ribose to the free amino groups of BSA or alkaline phosphatase (3000 units/mg). A coupling ratio of 11 mol cytokinin/mol of BSA was determined by spectrophotometry.

Immunization Protocol

The hapten conjugate (1.0 mg) was dissolved in 2.5 mL PBS buffer (50 mM Na_2HPO_4/NaH_2PO_4, 0.15 M NaCl, pH 7.2) and mixed with an equal volume of Freund's complete adjuvant. Four rabbits were immunized, each by four injections of freshly prepared antigen emulsion (0.4 mL equally divided into its foot pads), followed by multiple-site intradermal boostering with portions of about 100 μL of emulsion at days 0, 7, 28, 42, 72, 102, and 132. Blood was collected on the seventh day after the last injection and the immunoglobulin G fraction was precipitated with 50% saturated ammonium sulfate, lyophilized, and stored at −20°C.

ELISA

Assays of (oOH)[9R]BAP-type cytokinins were carried out on KOH-I-NOOR flat bottom plates (České Budějovice,

Czechoslovakia) using a modification of the ELISA described by Weiler *et al.* (27) for auxin. The wells were filled with 150 µL 50 mM $NaHCO_3$ (pH 9.6) containing 5 µg/mL immunoglobulin G, incubated overnight at 4°C for binding, and then washed twice with water to remove unbound antibody. To saturate remaining protein binding sites, the wells were filled with 200 µL of 0.02% (w/v) BSA in TBS buffer and incubated for 60 min at 4°C. After being rinsed with water, the coated wells were filled with 50 µL of TBS, 50 µL of sample or standard in TBS, and 50 µL of the cytokinin-alkaline phosphatase conjugate (0.1 µg/mL) in 0.02% (w/v) BSA in TBS and the plates were incubated at 4°C for 60 min. Unbound conjugates were removed by rinsing the plates four times with TBS buffer. The activity of bound alkaline phosphatase was determined using *p*-nitrophenylphosphate as a substrate (150 µL, 1 mg/mL in 50 mM $NaHCO_3$, pH 9.6, incubation for 60 min at 37°C). The reaction was terminated with 50 µL of 3 N KOH and the absorbance at 405 nm was read. Calibration of the ELISA was performed as described previously (21). Calculations of results were carried out as described by Weiler *et al.* (27). Sigmoidal standard curves for (oOH)[9R]BAP, cross-reacting compounds, dilution analysis, and internal standardization were linearized by the following transformation: logit B/Bo = ln(B/Bo)/(100-B/Bo) (see Fig. 1).

RESULTS

Sensitivity and Specificity of the (oOH)[9R]BAP ELISA

Antisera were raised in rabbits against the (oOH)[9R]BAP-BSA conjugate that had been prepared by the periodate method. Using the immunization protocol described above, antisera of high quality and comparable sensitivity and specificity were obtained (Table II). Because of the lowest degree of overall cross-reactivity, Ab 165 was routinely used for cytokinin analysis by ELISA. A typical standard curve obtained with Ab 165 and (oOH)[9R]BAP is shown in Figure 1. The inset shows the linearized curve providing a measuring range between 2.5 and 670 fmol/assay. Because as little as 3.05 fmol (1.14 pg) of (oOH)[9R]BAP could be detected, the assay was very sensitive. The midrange value (amount of antigen required for 50% inhibition) was 103 fmol for this cytokinin. Unspecific binding (binding in the presence of an excess of (oOH)[9R]BAP:313 pmol) was less than 3.5%. The coefficient of variation for the duplicate determination of standard B/Bo values within the measuring range was less than 4.0% in all of the ELISAs.

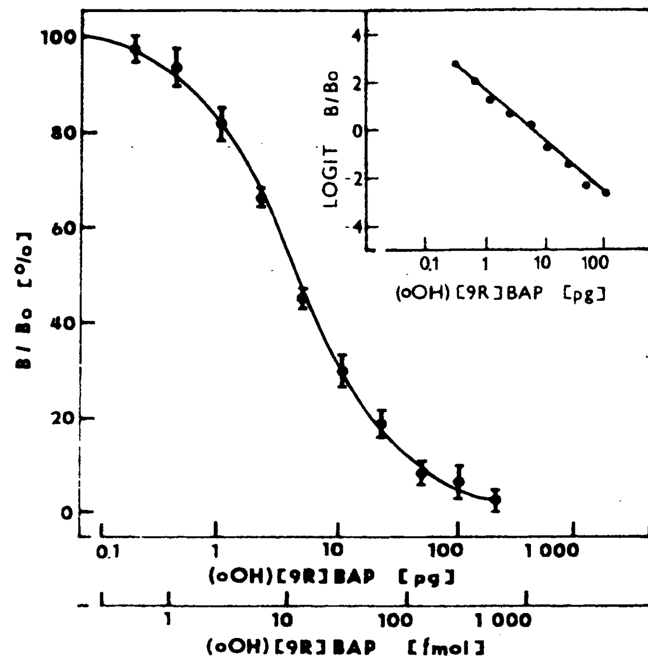

Figure 1. Standard curve for the (oOH)[9R]BAP ELISA and linearized logit/log plot of the same data (inset). Bars indicate SE (n = 20). B and Bo represent binding of alkaline phosphatase tracer in the presence and the absence of (oOH)[9R]BAP standard, respectively.

Specificity of polyclonal antibodies was determined by cross-reactivity studies (Table II). Cytokinins and other compounds that produced molar cross-reactions lower than 0.01% are not presented in Table II. Adenine, adenosine, inosine, and all natural isoprenoid cytokinins of the zeatin and dihydrozeatin group such as Z, [9R]Z, [9R-5'P]Z, (OG)[9R]Z, (OG)Z, [9G]Z, (diH)Z, (diH)[9R]Z, (diH)[9R-5'P]Z, (diH)[9G]Z, (diHOG)Z, (diHOG)[9R]Z, and the nonpurine cytokinin *N,N'*-diphenylurea, exhibited almost zero cross-

Table II. *Molar Cross-Reactivities of Several Cytokinins with (oOH)[9R]BAP Antibodies*

Data presented are expressed as percentage ratio of molar concentrations of (oOH)[9R]BAP and competitor producing 50% inhibition.

Compound	Antibody			
	164	165	166	167
(oOH)[9R]BAP	100	100	100	100
(oOH)BAP	50.50	24.61	11.11	31.82
(mOH)[9R]BAP	0.27	0.04	0.32	0.03
(mOH)BAP	0.20	0.02	0.20	0.02
[9R]BAP	0.67	0.09	1.50	0.18
BAP	0.39	0.08	0.60	0.14
[9R]iP	0.15	0.03	0.12	0.14
iP	0.09	0.04	0.08	0.08
[9R]K	0.10	0.04	0.20	0.13
K	0.06	0.03	0.10	0.10

reactivity. These compounds did not react even when present at 1 nmol/assay. [9R]iP and its free base showed at most a slight cross-reactivity. The same holds for the synthetic cytokinins kinetin and kinetin riboside. Interestingly, antibodies did not significantly bind cytokinins with unsubstituted or *m*-hydroxylated benzyl rings. The cross-reactivity data suggest that the *o*-position of the hydroxy group on the benzyl ring is one of the crucial requirements for immunoreactivity. Thus, hydroxylation in the *m*-position results in almost complete loss of immunoreactivity with (oOH)[9R]BAP antibodies. The low cross-reactivity with all tested cytokinins shows the high specificity of the four individually produced antibodies. On the other hand, the specificity with respect to the ribose moiety was low, as indicated by high immunoreactivity with the free base (oOH)BAP in all four ELISAs; its cross-reactivity was in the range between 11.1 and 50.5%.

Detection and Identification of (oOH)BAP in Poplar Leaves

The cross-reactivity studies showed that the ELISA used in this study provides a sensitive and specific assay for (oOH)[9R]BAP-type cytokinins. It was used to examine the occurrence of cytokinins structurally related to (oOH)[9R] BAP in plant tissues. Because the riboside has been identified in *Populus* × *Robusta* Schneid leaves (9, 10), this plant material (under the present scientific name *Populus* × *canadensis* Moench cv *Robusta*) was further investigated by the (oOH)[9R]BAP ELISA. The leaf extract was purified by butanol extraction and chromatography on cellulose phosphate, DEAE-cellulose, and octadecylsilica columns prior to HLPC and each fraction was assayed by ELISA. Approximately 95% of the immunoreactive material applied to the HPLC column was recovered in two peaks (Fig. 2). One peak coeluted with (oOH)[9R]BAP whereas the other, according to its retention time, could not be ascribed to any known cytokinin. With respect to its chromatographic behavior, this compound was tentatively interpreted as the free base (oOH)BAP. By acidic hydrolysis of (oOH)[9R]BAP, the free base was prepared and, upon HPLC, it coeluted with the unknown substance.

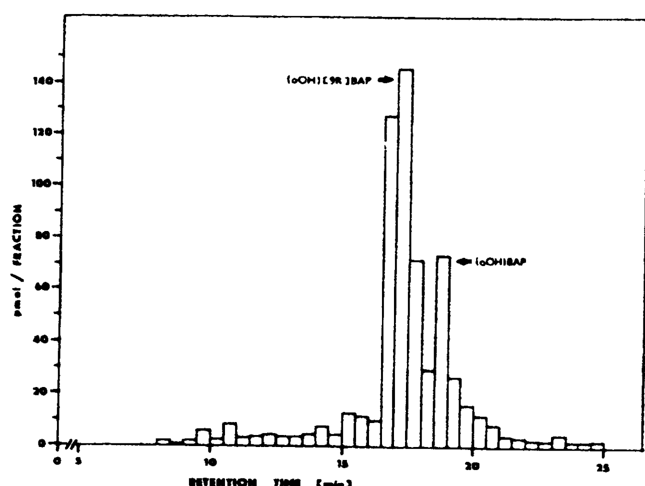

Figure 2. Immunoreaction with (oOH)[9R]BAP antiserum of compounds present in HPLC fractions of partially purified extracts from mature *P.* × *canadensis* Moench cv *Robusta* leaves. Chromatographic details and HPLC separation of different cytokinins have been described previously (21). Retention times of (oOH)[9R]BAP and (oOH)BAP standards are indicated by arrows.

ELISA was used in the purification procedure for isolation of substantial amounts of (oOH)BAP and its riboside for GC-MS analysis. Validation of the immunoassay data was achieved by serial dilution of the analyzed extracts and their HPLC fractionation with or without standard amounts of (oOH)BAP and (oOH)[9R]BAP. The diluted extracts analyzed by ELISA at different purification steps always produced parallel curves (Fig. 3A). Similarly, internal standardization of either crude, partially purified, or HPLC-fractionated extracts resulted in straight lines parallel to the standard curve (Fig. 3B). The HPLC fractions contained small amounts of immunoreactive material other than (oOH)[9R]BAP and its free base, which could not be ascribed to any of the cytokinin standards on the retention time basis (Fig. 2). The specificity of ELISA suggested that the unknown compounds may be structurally related to (oOH)[9R]BAP.

To confirm the presence of (oOH)BAP in the immunoreactive peak, the respective HPLC fraction was trimethylsilylated and subjected to GC-MS analysis. The TMS derivative produced a mass spectrum (Fig. 4) that was identical to that of an authentic standard. In addition the molecular ion of m/z 313 shows the expected mass of (oOH)BAP containing only 1 TMS group. The presence of fragment ions at m/z 135, 120, and 119 is indicative of an adenine fragment. Shannon and Letham (18) suggested ions of m/z 148 and 149 as a characteristic feature of an adenine substituted with a $-CH_2$-group at N^6. Hence, mass fragments at m/z 163, 179, and 194 presumably represent the remaining fragments of the side chain that must be of an aromatic nature. The loss of a trimethylsilylated hydroxyl group (Δm 89) from the molecular ion indicated the presence of a hydroxyl group on the benzyl ring (m/z 224). The m/z 385 ion arises from a di-TMS derivative with the additional TMS group attached to N^9 of adenine. Hence, the coincidence of the mass spectra of an authentic test substance and the unknown compound, as well as the characteristic mass and fragments, provide evidence for the chemical structure of the compound as N^6-(*o*-hydroxybenzylamino)purine.

Quantification of (oOH)[9R]BAP and (oOH)BAP in the Leaf Extracts

The content of (oOH)[9R]BAP and its free base/g fresh weight of *P.* × *canadensis* Moench cv *Robusta* leaf tissues was estimated by the ELISA using an internal standard [2-^{3}H](oOH)[9R]BAP (78% yield). The level of (oOH)[9R] BAP determined from five duplicate estimates was found to be 125.5 ± 13.4 ng/g fresh weight. The respective correction was made for (oOH)BAP on the basis of its molar cross-reactivity in the (oOH)[9R]BAP ELISA. The content of this cytokinin in mature leaves, as estimated by internal standardization, was 134 ± 18.3 ng/g fresh weight. The fraction containing this cytokinin exhibited the following UV spectral characteristics (λ_{max}): 80% (v/v) ethanol, 267.0; 0.02 N

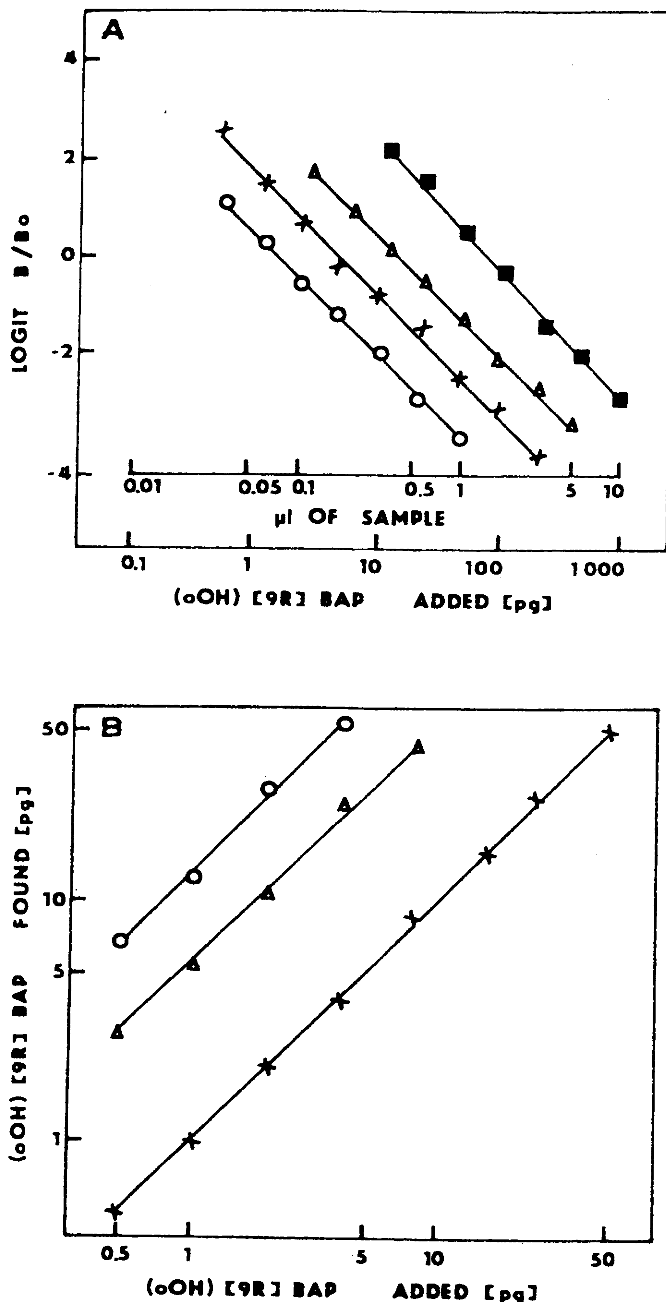

Figure 3. A, Logit transformation of ELISA standard curve for (oOH)[9R]BAP (×) and sample dilution curves for a *P.* × *canadensis* Moench cv *Robusta* extract at different purification steps: (O), crude extract; (Δ) ammonia eluate from cellulose phosphate; (■) HPLC fractions containing (oOH)[9R]BAP. B, Logit transformation of ELISA data using calibration standards (×) or a fixed amount (0.5 µL) of crude (O), or of partially purified (DEAE-cellulose-octadecyl silica chromatography) (Δ) extract of *P.* × *canadensis* Moench cv *Robusta* leaves after addition of different amounts of unlabeled (oOH)[9R]BAP.

NH_4OH in 80% (v/v) ethanol, 274.5; 0.1 N CH_3COOH, 273.5, which are indicative of a N^6-monosubstituted adenine (12).

DISCUSSION

Allowing quantification of as little as 3 fmol/assay, the ELISA described here is 1 order of magnitude more sensitive than comparable ELISAs developed for isoprenoid cytokinins (26). Similar sensitivity has been reported only with the solid-phase ELISA for [9R]iP using a detection system based on the high affinity between avidin and biotin (19). Our assay is highly specific for the N^6-substituent of (oOH)BAP and does not cross-react with the corresponding *m*-hydroxy compound or the unhydroxylated BAP. This strong discrimination was found with all four antisera and, hence, must be typical of this kind of antibody. High specificity for any substituent at N^6 was consistently found when the cytokinin hapten and protein (BSA) were conjugated via a spacer such as ribose. Nevertheless, the selectivity, as well as the high sensitivity of the ELISA described here, suggest that aromatic cytokinins provide better epitopes for antibody recognition than isoprenoid cytokinins (21, 22).

The high cross-reactivity between the antibodies raised against the riboside and the (oOH)BAP suggests that alterations at the adenine moiety have less impact on the intensity of the immunoreaction than changes of the N^6-substituent. Hence, in addition to the riboside and the free base, other (oOH)BAP derivatives (*e.g.* the 5′-monophosphate or N^9-glucosides or amino acid conjugates) may also cross-react with antibodies and thus presumably can be measured with the (oOH)[9R]BAP ELISA (14) if present in the same HPLC fraction. Unfortunately, such compounds were not available. On the other hand, glycosylation of the *o*-hydroxy group could change the hapten dramatically, rendering a cross-reaction with (oOH)[9R]BAP antisera very unlikely.

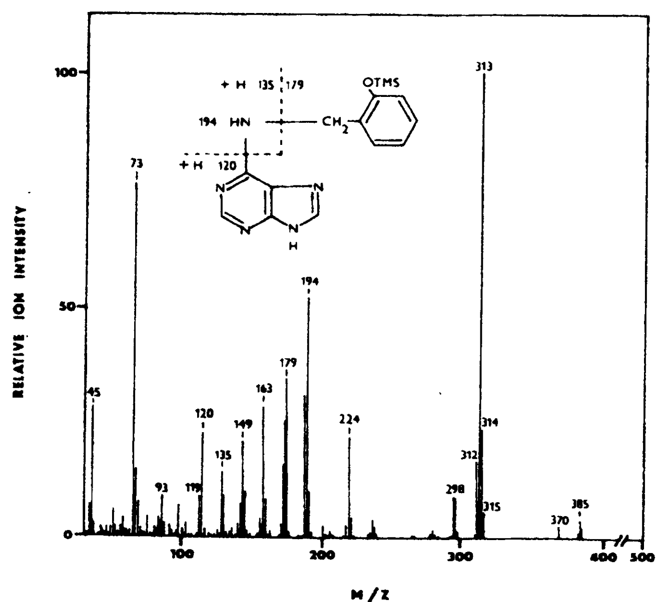

Figure 4. Mass spectrum of TMS derivative of the unknown aromatic cytokinin.

SUBSTITUENT			ABBREVIATION	REFERENCE
R_1	R_2	R_3		
$-CH_2-$	H	H	BAP	(16, 17, 22)
	H	Rib	[9R]BAP	(8, 16, 17, 22)
	H	Rib-5'-P	[9R-5'P]BAP	(16)
	H	Glc	[9G]BAP	(17)
$-CH_2-$ (OH)	H	H	(oOH)BAP	(p.c.)[a]
	H	Rib	(oOH)[9R]BAP	(4, 9, 10)
	CH_3S	Glc	(oOH)[2MeS 9G]BAP	(5)
$-CH_2-$ (OH)	H	H	(mOH)BAP	(22, 23)
	H	Rib	(mOH)[9R]BAP	(22, 23)

[a] p.c. means present communication

Figure 5. Naturally occurring aromatic cytokinins identified in plant species.

As a consequence of the high affinity between antigene and antibody, the ELISA described here is less prone to nonspecific interference with other compounds of a plant extract than other analytical procedures. This was demonstrated by validating the assay in a series of internal quality controls, including sample dilution analysis (Fig. 3A), internal standardization (Fig. 3B), and HPLC fractionation (Fig. 2). Even when the crude leaf extract was investigated, the ELISA characteristic was parallel to that produced with the pure cytokinin (Fig. 3A). Low interference with contaminants appears to be typical of most of the cytokinin ELISAs (1, 3, 6). With respect to the riboside of (oOH)BAP in poplar leaves, quantification by ELISA is in fair agreement with the results obtained previously with the soybean callus bioassay (10). However, our data support the findings of several laboratories that bioassays show somewhat lower cytokinin concentrations than GC-MS, radioimmunoassay, or ELISA (1). The reason for the apparently smaller amounts determined with the bioassay may be attributed to an eventual interference with inhibitory compounds in the extracts (7).

The identification of (oOH)BAP in *P.* × *canadensis* leaves (Fig. 4), together with the detection of several immunoreactive compounds in the HPLC fractions by ELISAs for (mOH)[9R]BAP and [9R]BAP (22), show that aromatic cytokinins presumably will occur in many more plant species than has been previously supposed. The structures of aromatic cytokinins identified so far in plant tissues are shown in Figure 5. Notably, BAP and its analogs constitute a distinct class of cytokinins occurring naturally in plants. Further studies of the chemical structure of compounds of that class are in progress.

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Enzyme Immunoassays of N^6-Benzyladenine and N^6-(*meta*-Hydroxybenzyl)adenine Cytokinins

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Abstract. Enzyme-linked immunosorbent assays (ELISAs) were developed for determination of N^6-benzyladenosine, N^6-(*meta*-hydroxybenzyl)adenosine, and structurally related cytokinins. The use of the ELISAs allowed detection over the range of 0.05–70 pmol for N^6-benzyladenine and 0.01–20 pmol for the N^6-(*meta*-hydroxybenzyl)adenine cytokinins. Polyclonal antibodies used in the assays were specific for N^6-benzyladenine and N^6-(*meta*-hydroxybenzyl)adenine and their corresponding N^9-substituted derivatives. By the use of internal standardization, dilution assays, authentic [2-^{3}H]cytokinin recovery markers, and immunohistograms, the ELISAs have been shown to be applicable for the estimation of N^6-benzyladenine and N^6-(*meta*-hydroxybenzyl)adenine-type cytokinins in plant tissues. For the analysis of cytokinins in the tissues of young poplar leaves and *Solanum* teratoma shoot culture, the extracts were fractionated by high performance liquid chromatography (HPLC) and the fractions analyzed by ELISAs. Immunohistogram ELISA analysis of fractions from different HPLC systems indicated major peaks of immunoreactivity co-chromatographing with the labeled and unlabeled standards of N^6-benzyladenine, N^6-*meta*-hydroxybenzyl)adenine, and their N^9-glycosides in these tissues.

Key Words. N^6-Benzyladenosine—Cytokinins—Enzyme immunoassay—N^6-(*meta*-Hydroxybenzyl) adenosine—*Populus* × *Robusta* leaves—*Solanum* shoots (transformed and normal plants)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FW, fresh weight; (mOH)[9*R*]BAP, N^6-(*meta*-hydroxybenzyl)adenosine; HPLC, high performance liquid chromatography; TBS, Tris-buffered saline; TEAA, triethylammonium acetate; [9*R*]BAP, N^6-benzyladenosine.

For the major groups of naturally occurring cytokinins, polyclonal and monoclonal antibodies of high quality have been developed (see Weiler 1984, Strnad et al. 1992a). Only a few studies have been carried out with cytokinins bearing an aromatic ring as the side chain. Constantinidau et al. (1978) described the production and immunologic characteristics of an antiserum against N^6-benzyladenosine, a synthetic cytokinin, which has already been identified in an old *Pimpinella anisum* cell culture (Ernst et al. 1983b) and *Solanum* crown gall tumors (Nandi et al. 1989a). For analysis of synthetic N^6-benzyladenine levels in plant tissue cultures, several workers have used the antibodies against isopentenyladenosine, which are known to cross-react strongly with [9*R*]BAP-type cytokinins (Ernst et al. 1983b, Vaňková et al. 1987, Label and Sotta 1988). We have already described an ELISA based on antibodies specific for N^6(*ortho*-hydroxybenzyl)adenosine, an aromatic cytokinin present at very high endogenous levels in *Populus* × *Robusta* leaves (Strnad et al. 1992b). In this paper I report the development of the enzyme immunoassays for detection and quantification of N^6-benzyladenosine ([9*R*]BAP), N^6-(*meta*-hydroxybenzyl)adenosine ((mOH)[9*R*]BAP), and related compounds. Using the ELISAs in conjunction with different reversed phase HPLC separations, it was possible to detect several immunoreactive compounds coeluting with authentic labeled and unlabeled standards of aromatic cytokinins in young poplar leaves (*Populus* × *canadensis* Moech., cv. *Robusta*) and teratoma shoot culture derived from *Solanum* leaf discs transformed by T-DNA gene 4 (*ipt*).

Materials and Methods

Chemicals and Reagents

Unlabeled cytokinins were from Apex Organics (Leicester, UK); isopentenyladenosine, isopentenyladenine, zeatin, zeatin riboside, dihydrozeatin; dihydrozeatin riboside, kinetin, N^6-benzyladenosine, N^6-

benzyladenine, and N,N'-diphenylurea were from Sigma (St. Louis, MO, USA); N^6-(hydroxybenzyl)adenines and their ribosides were kindly supplied by Dr. Tomáš Vaněk, Institute of Organic Chemistry and Biochemistry, Prague. N^6-(*ortho*-Hydroxybenzylamino)-N^9-β-D-glucopyranosylpurine, N^6-(*meta*-hydroxybenzylamino)-N^9-β-D-glucopyranosylpurine, N^6-[2-^{3}H]benzyladenine, N^6-[2-^{3}H] benzyladenosine, N^6-[2-^{3}H](*meta*-hydroxybenzyl)adenine, and N^6-[2-^{3}H](*meta*-hydroxybenzyl)adenosine (specific activity approximately 1.0 TBq · mmol^{-1}) were synthesized by Dr. J. Hanuš, Isotopic Laboratory, Institute of Experimental Botany, Prague, by an unpublished method. Before use for syntheses, analyses, and cross-reactivity studies, the purity of labeled and unlabeled cytokinins was checked by HPLC. Alkaline phosphatase for enzyme immunoassay (2,500 units · mg^{-1}) and *p*-nitrophenylphosphate were from Boehringer (Mannheim, FRG); acetonitrile for chromatography was from Merck (Darmstadt, FRG); Tris, bovine serum albumin, and acid phosphatase (0.4 units · mg^{-1}) were from Sigma, DEAE-cellulose, a reversed phase column (Separon SGX C_{18}), and C_{18} cartridges were from Tessek (Prague, Czech Republic). All other chemicals were obtained from Lachema (Brno, Czech Republic).

Plants

The growing leaves of poplar (*P.* × *canadiensis* Moench., cv. *Robusta*), collected from the field on June 10, were used for cytokinin analysis. The first four young leaves without petioles were cut just 1 h after daybreak, dropped immediately into liquid nitrogen, and extracted. Potato shoots (*Solanum tuberosum* L., cv. *Oreb*) grown on Murashige and Skoog (1962) medium without cytokinin were either control plants or teratoma shoots (clone 1). Clone 1 was selected after transformation of *Solanum* leaf discs by pTi C58 T-DNA gene 4 (*ipt*) and formed moss-like teratomas (Ondřej et al. 1990). The shoots were collected 4 weeks after subcultivation and then either extracted or stored at −70°C until use.

Immunologic Reagents

N^6-Benzyladenosine and N^6-(*meta*-hydroxybenzyl)adenosine were coupled to bovine serum albumin by a modification of the method of Erlanger and Beiser (1964). Cytokinin (30 μmol) was dissolved in a solution of 200 μl of dimethyl sulfoxide and 2 mL of bidistilled water, and 2 mL of 0.03 M $NaIO_4$ solution (60 μmol) was added dropwise over a period of 5 min. The solution was stirred for 15 min in the dark at room temperature. The excessive periodate was destroyed by adding 15 μL of 1.8 M ethylene glycol (30 μmol). After 5 min the reaction mixture was added in portions of 50 μL to bovine serum albumin dissolved in carbonate buffer (10 mM K_2CO_3, 10 mM $KHCO_3$, pH 9.6). The solution was stirred at 4°C for 60 min in the absence of light. During this period the pH was kept between 9.3 and 9.5 with 5% K_2CO_3. The conjugates were stabilized overnight at 4°C with an excess of $NaBH_4$ (5 mg, 132 μmol), then dialyzed against 6 × 2 liters of phosphate-buffered saline (50 mM, $NaHPO_4$, 0.15 M NaCl, 0.4 g liter^{-1} NaN_3, pH 7.4), lyophilized, and stored at −20°C. From the UV spectra of the conjugates a coupling ratio of 9 mol of (mOH)[9*R*]BAP and 7 mol of [9*R*]BAP/mol of bovine serum albumin was determined. The immunization schedule and purification of antibodies are described in detail in our previous papers (Strnad et al. 1990, 1992b).

Extraction and Purification of Cytokinins from Plant Tissues

The procedure for tissue extraction and purification is a modification of the method described previously by MacDonald et al. (1981). Frozen plant tissues were ground to a fine powder under liquid nitrogen. The powder was divided into three aliquots corresponding to 2 g fresh weight (FW). Each aliquot was extracted in ice-cold 80% methanol(10 mL · g^{-1} FW) containing sodium diethyldithiocarbamate as antioxidant (400 μg · g^{-1} FW). About 420 Bq (25,000 dpm) of [2-^{3}H](mOH)[9*R*]BAP, [2-^{3}H][9*R*]BAP, and corresponding tritiated free bases were added to the extracts to monitor for losses during purification steps and to validate the chromatographic data. After a 2-h extraction, the homogenate was centrifuged (15,000 × g, 4°C) and pellets reextracted the same way. The combined extracts were concentrated to approximately 1.0 mL by rotary evaporation under vacuum at 35°C. The samples were diluted to 20 mL with ammonium acetate buffer (40 mM, pH 6.5) containing sodium diethyldithiocarbamate (5 mM) and then incubated with wheat germ acid phosphatase (0.05 units · mL^{-1}) for 30 min in the dark (25°C) to dephosphorylate cytokinin 5′-phosphates. For the immunoassay dilution analysis, the 2-mL eluates were dried in vacuo and redissolved in Tris-buffered saline (TBS, 50 mM Tris, 10 mM NaCl, 1 mM $MgCl_2$, pH 7.5). Aliquots of these solutions were either analyzed in serial dilutions or mixed with known amounts of cytokinin standards and then analyzed by ELISA.

The extracts were purified using a combined DEAE-cellulose (1.0 × 5.0 cm)-octadecylsilica (0.5 × 1.5 cm) column as described in MacDonald et al. (1981). Cytokinins were loaded onto a reversed phase C_{18} column cartridge that was then washed with 10 mL of H_2O and eluted in 7 mL of 70% (v/v) methanol in triethylammonium acetate buffer (TEAA, 40 mM, pH 3.35). The eluates were evaporated to dryness, dissolved in 0.5 mL of 70% methanol in TEAA (7:3 v/v), and filtered through a Millipore filter (0.22 μm).

High Performance Liquid Chromatography

The equipment consisted of a Spectra Physics SP 8800 solvent delivery system coupled to an SP 100 UV-vis detector and SP 4400 computing integrator. The injection was performed by a Rheodyne 7010 injection loop (100 μL). Two different gradient systems on a Separon SGX C_{18} column (250 × 4 mm inner diameter, 7 μm particle size; Tessek) were used to separate different aromatic cytokinins. In system I solvent A was 20% methanol in TEAA buffer (v/v, 40 mM, pH 3.35); solvent B 80% methanol in 40 mM acetic acid (v/v, pH 3.65). Initial conditions were 90% A, 10% B; then a linear gradient to 60%A, 40% B at 15 min; a linear gradient to 40% A, 60% B at 24 min; 100% methanol for 10 min (column wash); and 90% A, 10% B at 10 min (regeneration). The flow rate was 1.0 mL/min. In system II, the column was eluted at 1.2 mL/min with acetonitrile and TEAA buffer (40 mM, pH 3.35) according to the following gradient profile: 0 min of 5% acetonitrile, 10 min of 7%, 30 min of 10%, 40 min of 15%, 50 min of 14%, 10 min of 100% (washing). Timed fractions (0.5 min) were collected by a FRAC 100 fraction collector (Pharmacia, Uppsala, Sweden), dried in vacuo, and redissolved in 500 μL of TBS buffer. Fifty-μl aliquots were investigated in duplicate by scintillation counting and ELISA. The content of individual cytokinins in the appropriate immunoreactive fractions was assessed using a series of different ELISAs including dilution and internal standardization (Weiler 1982, Badenoch-Jones et al. 1984).

Enzyme-linked Immunosorbent Assay (ELISA)

The assays were performed using a modification of the ELISA protocol described by Weiler et al. (1981). The microtiter plates (Gama, České Budějovice, Czech Republic) were coated with 150 μL of rabbit anti-[9*R*]BAP or anti-(mOH)[9*R*]BAP antibodies (5 μg · mL^{-1} 50 mM $NaHCO_3$, pH 9.6). The wells were washed with distilled water, filled with 200 μL of bovine serum albumin solution (0.04 g · L^{-1}), and incubated for 1 h at 25°C. After decanting and two washes with dis-

Table 1. Assay parameters of N^6-benzyladenosine and N^6-(*meta*-hydroxybenzyl)adenosine enzyme immunoassay.

Parameter	[9R]BAP assay	(mOH)[9R]BAP assay
Amount of tracer/assay	5 ng	2 ng
Unspecific binding	3.5%	2.2%
Detection limit	76 fmol, 27 pg	19 fmol, 7 pg
Linear average of logit/log plot	0.05–70 pmol	0.01–20 pmol
Midrange (50% binding)	1.5 pmol, 0.54 ng	0.5 pmol, 190 pg
Intraassay variance[a]	4.2%	3.5%
Interassay variance[b]	6.8%	6.1%

[a] Eight replicates.
[b] Twenty assays.

tilled H_2O, the wells were filled in the following sequence: 50 μL of TBS, 50 μL of standard or sample in TBS, and 50 μL of cytokinin-alkaline phosphatase tracer diluted in TBS-bovine serum albumin buffer (0.04 g · L^{-1}). Nonspecific binding was determined by adding an excess (200 pmol) of cytokinin standard; for maximum tracer binding, TBS was used instead of standard. After 1 min of shaking, the plates were incubated for 1 h at 25°C. The decanted plates were then washed four times with TBS and filled immediately with 150 μL of a *p*-nitrophenylphosphate solution (1 mg · mL^{-1} 50 mM $NaHCO_3$, pH 9.6). The reaction was stopped after a 1-h incubation at 25°C by adding 50 μL of 5N KOH and the absorbance measured at 405 nm in a Titertek Multiscan MCC 340 (Flow Laboratories, Irvine, UK). A Wia-Calc computer program (LKB, Bromma, Sweden) was used for assay evaluation and computation of results. Sigmoidal curves for standards, cross-reacting compounds, and dilution analysis were linearized by log-logit transformation as follows (Weiler 1980): logit B/B_0 = ln $[(B/B_0)/(100 - B/B_0)]$ (see *insets*, Fig. 1).

The cytokinin value obtained by the ELISA of the fraction(s) containing 2-^{3}H-labeled cytokinin was corrected by the appropriate cross-reactivity and recovery values to obtain estimates of cytokinin levels in plant tissue (expressed as [9*R*]BAP or (mOH)[9*R*]BAP equivalent). If there was any spread of radioactivity into a second fraction, the cytokinin content was estimated from the fractions containing radioactivity. Levels of N^9-glucosides were calculated from immunoactivity (in the appropriate ELISA) of fraction(s) collected at the retention time of authentic standards and on the assumption that recovery for these cytokinins was same as the recovery of 2-^{3}H-labeled ribosides.

Results

Assay Characteristics

All immunized rabbits produced antisera to the cytokinin conjugates, but serum titers differed considerably and reflected the reaction of the individual animal. Because of its high selectivity characteristics, antibody 474 specific for [9*R*]BAP and antibody 754 specific for (mOH)[9*R*]BAP were selected and used routinely for cytokinin analysis. Some of the assay parameters are summarized in Table 1. The mean standard curves and their log/logit plots are shown in Fig. 1. The dilutions of antisera required to give 50% binding of an appropriate 2-^{3}H-labeled cytokinin were 1:28,000 and 1:150,000 for [9*R*]BAP and (mOH)[9*R*]BAP, respectively. As little as 76 fmol of [9*R*]BAP and 19 fmol of (mOH)[9*R*]BAP could be detected by the ELISAs. Within the measuring range, the standard curves were almost linear over 3 orders of magnitude with small inter- and intraassay variation.

The specificity of antibodies was determined by cross-reactivity studies, and the results are shown in Table 2. The compounds were tested for antibody binding over a range from 0.01 up to 5,000 pmol/assay. Data for cytokinins and related compounds producing molar cross-reactivities lower than 0.01% are not shown, namely, no cross-reactivity was found for adenine, adenosine, adenosine 5′-monophosphate, inosine, *N,N*′-diphenylurea, zeatin 7-glucoside, dihydrozeatin 7-glucoside, dihydrozeatin 9-glucoside, and *O*-glucosides of zeatin and dihydrozeatin even when tested in amounts up to 5,000 pmol/assay. Other natural isoprenoid cytokinins such as zeatin, zeatin riboside, zeatin 9-glucoside, zeatin riboside 5′-monophosphate, *cis*-zeatin, *cis*-zeatin riboside, dihydrozeatin, and dihydrozeatin riboside showed at most only slight cross-reactivity. In addition to the riboside, the antibodies cross-reacted strongly with respective free bases, riboside 5′-monophosphates, and N^9-glucosides. The slopes of the log/logit transformation of all N^9-substituted derivatives were similar to the standard curves of [9*R*]BAP and (mOH)[9*R*]BAP, respectively (data not shown).

Surprisingly, there was a very low level of competition by N^6-benzyladenine, N^6-(*ortho*-hydroxybenzyl)adenine, and their N^9-glycosides for antibodies raised against *meta*-derivative. Thus, the position of the hydroxyl group on the benzyl ring is a crucial factor for antibody recognition. As expected, the anti-[9*R*]BAP antibodies were reactive with isopentenyladenine, kinetin, and their N^9-glycosides because of the apolar side chain of the original antigen. Interestingly, this antibody also bound appreciably benzyladenine 3-glucoside and N^6-(*meta*-hydroxybenzyl)adenine and its N^9-substituted derivatives. In consequence, by replacing of [9*R*]BAP with (mOH)[9*R*]BAP tracer, the assay is also suitable for [9*R*]BAP analysis.

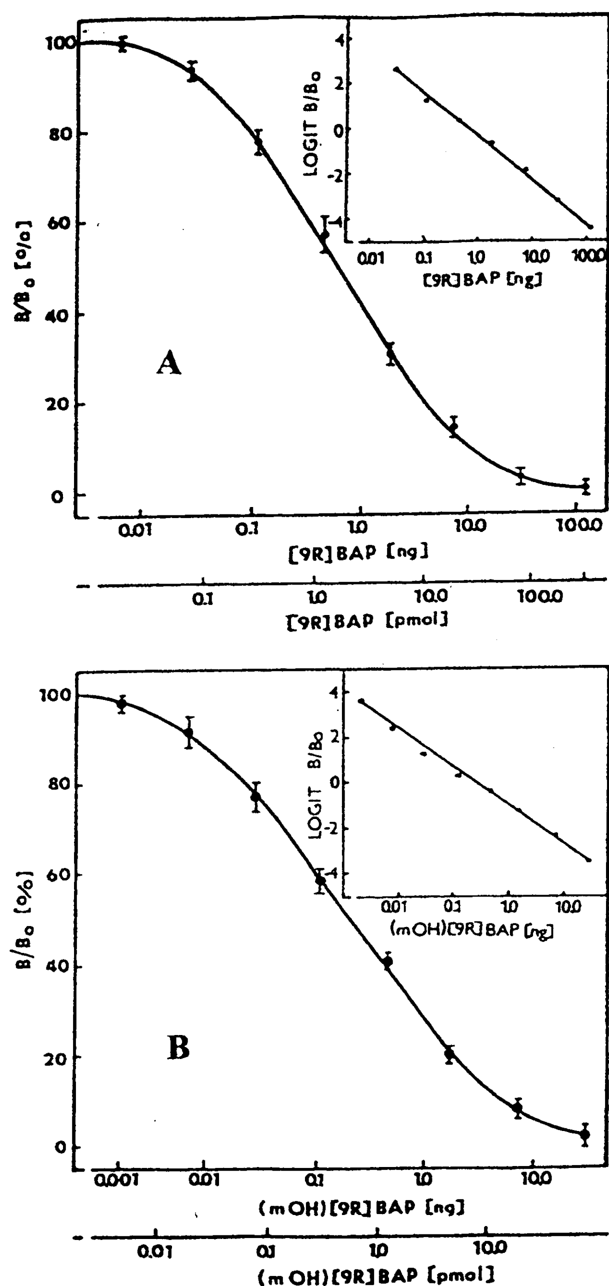

Fig. 1. Typical standard curves obtained for [9*R*]BAP and (mOH)[9*R*]BAP ELISAs and linearized logit/log plot of the same data (inset). Bars indicate standard deviation of duplicates ($n = 20$); B and B_0 represent binding of alkaline phosphatase tracer in the presence and absence of [9*R*]BAP and (mOH)[9*R*]BAP, respectively.

Validation of the ELISAs

Validation of assay performance at different purification steps was carried out to assess the reliability of the ELISAs. Details for one of the sampled (*P.* × *Robusta*) are shown in Fig. 2. When serially diluted crude extracts were analyzed by ELISA, parallel curves were always obtained (Fig. 2), suggesting that these samples did not contain substances interfering with the assays. When activity was detected in HPLC fractions that were assayed at more than one dilution, the dilution curves were also parallel to the standard curves (Fig. 2). Similarly, the recoveries of internal standards added to the crude and HPLC-purified extracts were found to produce satisfactory parallel lines (Fig. 2). Accurate quantification of the cytokinins in plant extracts was performed by ELISA of HPLC-purified extracts in conjunction with recoveries of internal radiolabeled standards. Recoveries of 83% for [2-^{3}H](mOH)[9*R*]BAP and 76% for [2-^{3}H][9*R*]BAP were obtained, whereas the recoveries of corresponding free bases were 68 and 63%, respectively. Tritium-labeled cytokinins used in this study proved also to have a useful application in locating the HPLC fractions containing immunoreactive cytokinins. Thus, any possible spread of the immunoactivity into neighboring fractions could be detected and accounted for, based on these recovery markers.

Immunodetection and Quantification of Cytokinins in Plant Extracts

The broad specificity of the antibodies for N^9-substituents of aromatic cytokinin allowed these forms to be quantified together with those for which the assays were developed. This was achieved by separating all cross-reactive compounds using two different HPLC systems on the Separon SGX C_{18} column (Fig. 3). This column is unique in that it separates cytokinins in methanolic gradient (system A) according to their apparent hydrophobicity; but when separated in system B (acetonitrile-TEAA buffer as a solvent), the N^9-glucosides eluted first followed by the free bases, and the ribosides were retained most strongly among their corresponding N^9-substituted derivatives (Fig. 3*B*). Furthermore, there was a good separation of aromatic cytokinins from isoprenoid ones (see Strnad et al. 1990, Jones et al. 1996). However, the batch-to-batch variability in the stationary phase was quite high.

Assay of HPLC-purified extract of *P.* × *Robusta* leaves with the ELISA for (mOH)[9*R*]BAP detected cross-reactive compounds coeluting with those of authentic and labeled (mOH)[9*R*]BAP and its free base (Fig. 4). The values obtained from two HPLC systems of the three duplicate estimates were 20.8 + 3.4 and 7.2 ± 0.9 pmol · g^{-1} FW, respectively. ELISA using anti-[9*R*]BAP antibodies revealed peaks corresponding to N^6-benzyladenosine (0.68 ± 0.12 pmol · g^{-1} FW) and N^6-benzyladenine (0.31 ± 0.09 pmol · g^{-1} FW). In addition, the anti-[9*R*]BAP antibodies cross-reacted with a compound that in the methanolic gradient had a retention time of 13.8 min (Fig. 4). It was deduced to be

Table 2. Molar cross-reactivities of various cytokinins with N^6-benzyladenosine and N^6-(*meta*-hydroxybenzyl)adenosine antibodies. Data presented are expressed as the percentage ratio of molar concentration of [9R]BAP or (mOH)[9R]BAP and competitor giving 50% binding.

	Cross-reactivity (%)	
	Anti-[9R]BAP	Anti-(mOH)[9R]BAP
N^6-Benzyladenosine	100	0.07
N^6-Benzyladenine	72.2	0.05
N^6-Benzyladenine 3-glucoside	23.3	<0.01
N^6-Benzyladenine 7-glucoside	0.1	<0.01
N^6-Benzyladenine 9-glucoside	79.5	0.05
N^6-Benzyladenosine 5′-monophosphate	86	0.08
N^6-(*meta*-Hydroxybenzyl)adenosine	6.8	100
N^6-(*meta*-Hydroxybenzyl)adenine	2.8	43.4
N^6-(*meta*-Hydroxybenzyl)adenine 9-glucoside	4.7	68.7
N^6-(*ortho*-Hydroxybenzyl)adenosine	0.04	0.06
N^6-(*ortho*-Hydroxybenzyl)adenine	<0.01	0.03
N^6-(*ortho*-Hydroxybenzyl)adenine 9-glucoside	<0.01	0.04
Zeatin riboside	0.07	<0.01
Zeatin	0.05	<0.01
Zeatin 9-glucoside	0.06	<0.01
Zeatin riboside 5′-monophosphate	0.04	<0.01
cis-Zeatin riboside	0.09	<0.01
cis-Zeatin	0.06	<0.01
Dihydrozeatin riboside	0.09	<0.01
Dihydrozeatin	0.04	<0.01
Dihydrozeatin riboside 5′-monophosphate	0.03	<0.01
Isopentenyladenine	2.33	<0.01
Isopentenyladenine 9-glucoside	1.22	<0.01
Isopentenyladenosine 5′-monophosphate	2.12	<0.01
Kinetin riboside	2.53	<0.01
Kinetin	1.72	<0.01

(mOH)[9*R*]BAP because the dame fraction gave a high level of activity in the (mOH)[9*R*]BAP assay. Identity of the peak was confirmed by coelution of authentic radioactive and immunoactive compound on Microsorb C_{18} (Rainin) column and by mass spectrometry (Strnad et al. 1997). Furthermore, the amount of (mOH)[9*R*]BAP found after correction for cross-reactivity (6.8% in [9*R*]BAP assay) was approximately the same as that determined in the appropriate ELISA.

Fig. 5 shows the immunohistograms of the extracts from transformed and untransformed *Solanum* shoots analyzed by HPLC-ELISAs. Control potato shoots cultivated in vitro contained amounts of [9*R*]BAP and (mOH)[9*R*]BAP cytokinins too low to detect by this method (detection limit 0.2 pmol $\cdot$ g^{-1} FW), whereas a 4-week-old teratoma shoot culture showed considerably higher levels of (mOH)[9*R*]BAP and benzyladenine 9-glucoside (Fig. 5, *B* and *D*). The peak that eluted before benzyladenine 9-glucoside is almost certainly due to the immunoactivity of (mOH)[9*R*]BAP in the [9*R*]BAP assay as described above, e.g. it coeluted at the retention time of an authentic standard (labeled and unlabeled), and the tissue content (19.8 ± 1.7 pmol $\cdot$ g^{-1}FW) calculated from its cross-reactivity was similar to that obtained in the (mOH)[9*R*]BAP ELISA (see Fig. 5).

Discussion

The ELISAs for N^6-benzyladenosine and N^6-(*meta*-hydroxybenzyl)adenosine described here have a slightly higher sensitivity than the immunoassays for isoprenoid cytokinins described previously (Hansen et al. 1984, Barthe and Stewart 1985, Cahill et al. 1986, Eberle et al. 1986). Cross-reactivity data revealed that as for other cytokinin antibodies (Weiler 1980, Badenoch-Jones et al. 1984, Turnbull and Hanke 1985) there is a marked selectivity for features of the N^6-side chain, e.g. the presence of a polar group and its position on the side chain but lack of specificity for N^9-substituents (Strnad et al. 1992a). The cross-reactivities of amino acid conjugates are unknown, but they may cross-react and thus presumably could be measured by ELISAs (Badenoch-Jones et al. 1987b). The binding of benzyladenine 7-glucoside to anti-[9*R*]BAP antibodies, as expected, was low (see Badenoch-Jones et al. 1984), but the 3-glucoside was highly immunoactive. Unfortunately, *meta*-hydroxybenzyladenine 3-glucoside was not available for testing in the corresponding assay. In general, other workers have not determined the cross-reactivity of their antibodies with 3-glucosides, but the antiserum against isopentenyladenosine developed by Weiler and Spanier (1981) was

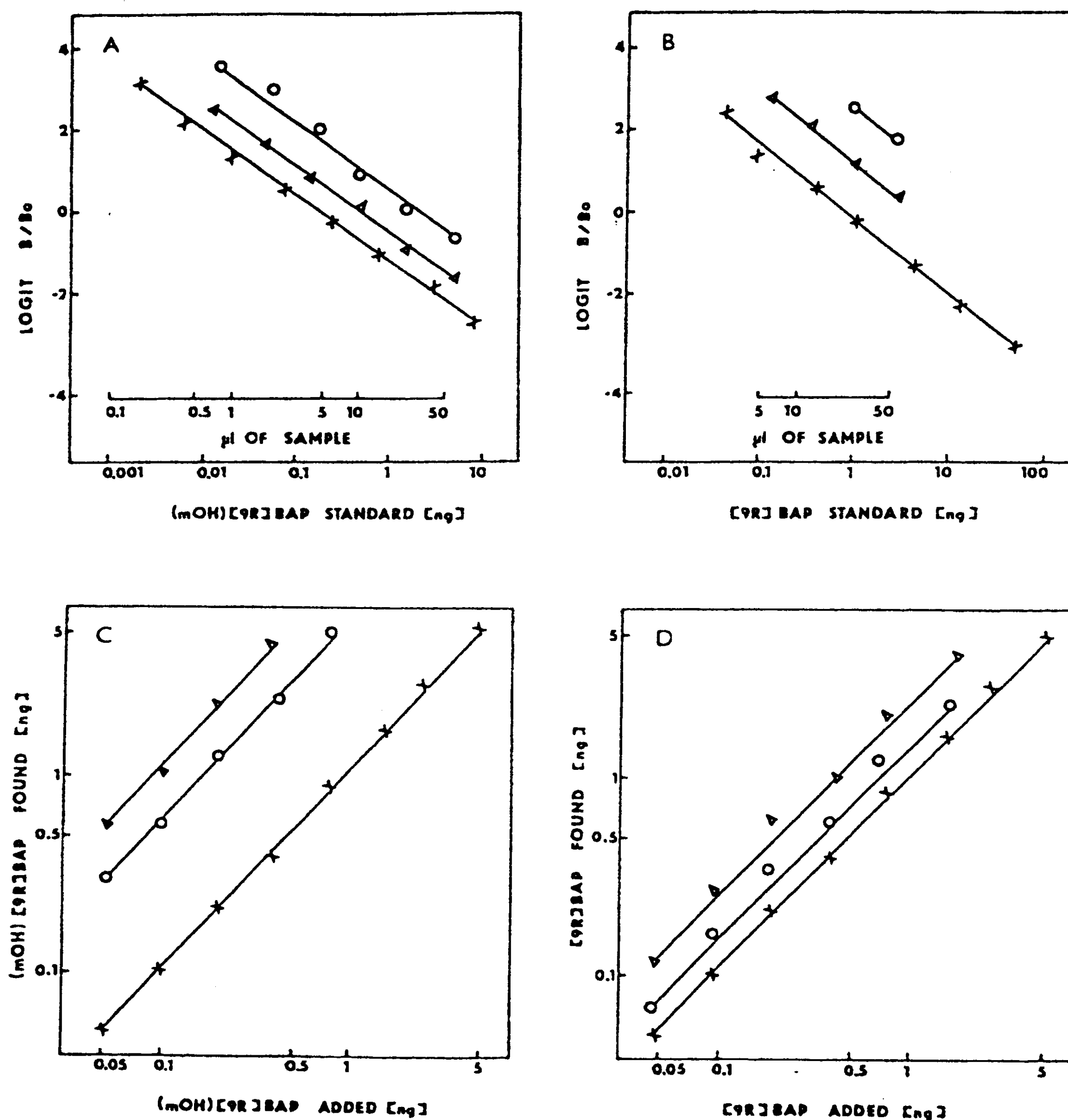

Fig. 2. Validation of the ELISA data for [9*R*]BAP (**right**) and (mOH)[9*R*]BAP (**left**). **A** and **B**, logit transformation of ELISA standard curves (×) and dilution curves of crude extract (○) and HPLC fractions containing corresponding cytokinin (▽). **C** and **D**, internal standardization using different amounts of unlabeled standard added to a fixed amount (50 µL) of crude (○) or HPLC-purified (▽) extract and comparison with the calibration line (×). Logit B/B_0 = $\ln[(B/B_0)/(100 - B/B_0)]$.

reported to show high cross-reactivity with benzyladenine 3-glucoside. The antibodies raised against hydroxylated benzyladenines are highly specific for the N^6-substituent (Strnad et al. 1992b), less so in the case of antibodies to [9*R*]BAP. As with the antibodies against isopentenyladenosine (Ernst et al. 1983a, Sotta et al. 1987) the antibodies against [9*R*]BAP showed moderate cross-reactivity against cytokinins bearing an apolar N^6-side chain such as kinetin, isopentenyladenine, and their N^9-substituted derivatives. The reasons for the high cross-reactivity of *meta*-hydroxybenzyladenines are not yet clear. However, the same degree of cross-reactivity toward (mOH)BAP-type cytokinins was obtained with anti-[9*R*]BAP antibodies raised against 5′-hemisuccinyl and 2′,3′-acetyl [9*R*]BAP derivatives (Siglerová and Strnad 1996, unpublished). The question arises as to whether the benzyl ring of [9*R*]BAP in the antigen might not have been specifically hydroxylated in the *meta*-position during immunization.

To demonstrate the applicability of the assays for the analysis of [9*R*]BAP and (mOH)[9*R*]BAP, young poplar leaves (*P.* × *canadensis* Moech., cv. *Robusta*) and trans-

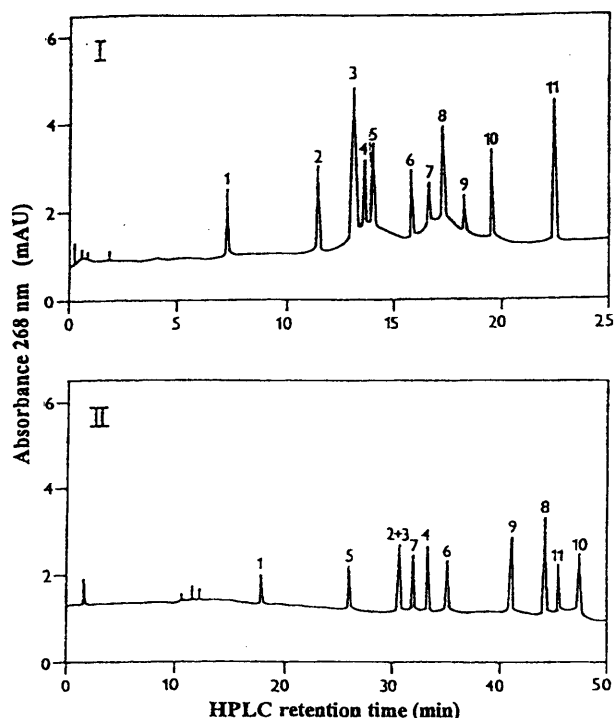

Fig. 3. Reversed phase HPLC separation of aromatic cytokinin standards. Column: 250 × 4.0 mm inner diameter, 7 µm Separon SGX C_{18}; detector at 268 nm. System A: flow rate, 1.0 mL/min, gradient between methanol-TEAA buffer (40 mM, pH 3.35) was 0 min 26%, 15 min 44%, 25 min 56%. System B: gradient between acetonitrile-TEAA buffer (40 mM, pH 3.35) was 0 min 5%, 10 min 7%, 30 min 10%, 40 min 15%, 50 min 14%. Peak numbers: **1,** N^6-(*meta*-hydroxybenzyl)adenine 9-glucoside; **2,** N^6-(*ortho*-hydroxybenzyl)adenine 9-glucoside; **3,** N^6-(*meta*-hydroxybenzyl)adenosine; **4,** N^6-benzyladenine 9-glucoside; **5,** N^6-(*meta*-hydroxybenzyl)adenine; **6,** N^6-(*para*-hydroxybenzyl)adenosine; **7,** N^6-(*para*-hydroxybenzyl)adenine; **8,** N^6-(*ortho*-hydroxybenzyl)adenosine; **9,** N^6-(*ortho*-hydroxybenzyl)adenine; **10,** N^6-benzyladenosine; **11,** N^6-benzyladenine.

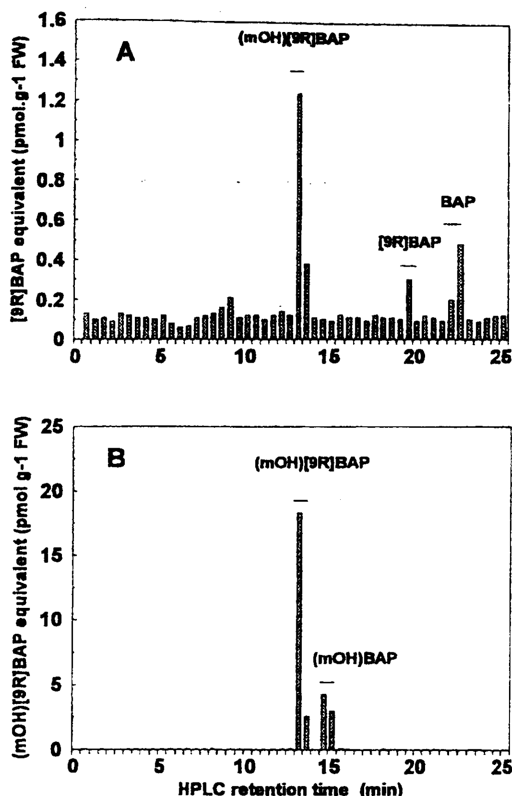

Fig. 4. Immunodetection of aromatic cytokinins in HPLC-fractionated extracts from young *P.* × *canadensis* Moench., cv. *Robusta* leaves by ELISAs for N^6-benzyladenosine (**A**) and N^6-(*meta*-hydroxybenzyl)adenosine (**B**). HPLC conditions for separation of aromatic cytokinins in methanolic gradient are as in Fig. 3a. Retention times of 2-^{3}H-labeled standards are indicated by **horizontal bars.**

formed *Solanum* plants known to contain aromatic cytokinins (Horgan et al. 1973, Strnad et al. 1992b, Nandi et al. 1989a) were analyzed. Interference in the assay by other compounds in the extracts, as indicated by nonparallelism of the standard curve and sample dilution curves, proved not to be a problem for any sample, even when crude extracts were analyzed by ELISAs. In addition, spiking with authentic standards for internal standardization of either crude extracts or HPLC fractions containing immunoactive substances produced parallel lines, as generally reported for cytokinin immunoassays (Weiler 1980, Badenoch-Jones et al. 1984, Eberle et al. 1986). Moreover, HPLC immunohistograms showed single peaks of immunoreactivity cochromatographing with the corresponding labeled and unlabeled standards. In spite of this evidence supporting the validity of the HPLC-ELISA, the identification of individual compounds in plant extracts by the method should be regarded as tentative for the following three reasons. First, the large number of different cytokinins in extracts makes it difficult to resolve them all unambiguously, especially by collecting fractions. Second, retention times in HPLC are subjected to slight variation due to impurities in the extract, fluctuating temperature, etc. Third, in some cases the levels of individual cytokinins can be extremely high, leading to appreciable immunoreactivity even in ELISAs in which such compounds show low cross-reactivity. It has been suggested that the immunoassays could be used in conjunction with appropriate systems for cytokinin separation (MacDonald et al. 1981, Badenoch-Jones et al. 1984). Clearly, it is important to separate all cytokinin metabolites even when cross-reactivities are lower than 0.1%. For the samples examined by [9*R*]BAP ELISA in the present study, a rechromatographing the putative *meta*-hydroxybenzyladenine-like cytokinins confirmed this (see Fig. 4). Furthermore, we have already detected very high N^6-(*ortho*-hydroxybenzyl)adenosine levels (as much as 1.0

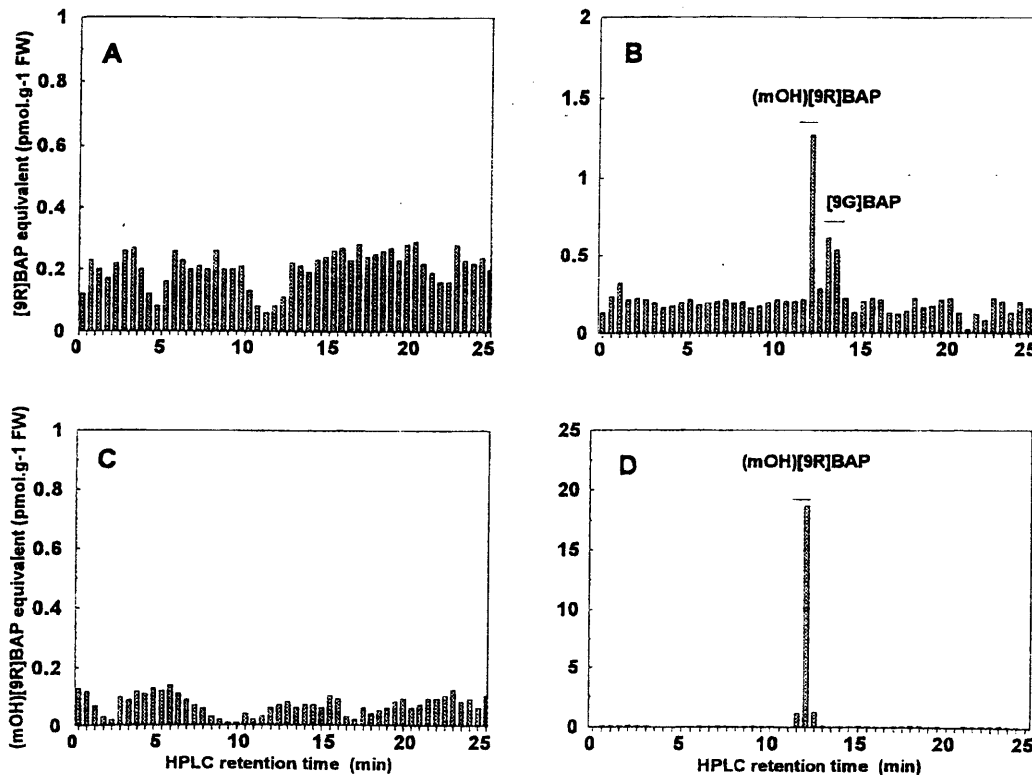

Fig. 5. Immunodetection of aromatic cytokinins in HPLC-fractionated extracts in untransformed (A and C) and transformed (B and D) and potato shoots by T-DNA gene 4 (*ipt*) by ELISAs for N^6-benzyladenosine (A and B) and N^6-(*meta*-hydroxybenzyl)adenosine (C and D). HPLC conditions for separation of aromatic cytokinins in methanolic gradient are as in Fig. 3a. Retention times of authentic and 2-^{3}H-labeled standards are indicated by **horizontal bars.**

nmol · g^{-1} FW) in HPLC-purified extracts either by appropriate assay (Strnad et al. 1992b) or by ELISAs for zeatin riboside, dihydrozeatin riboside, isopentenyladenosine, N^6-benzyladenosine, and N^6-(*meta*-hydroxybenzyl)adenosine (data not shown). Immunoassays developed in the present study proved to have useful application in locating and estimating the aromatic cytokinin-like substances in HPLC fractions. One method for achieving this in the presence of a high UV background was based on cochromatography of immunoactive substances with authentic radiolabeled standards and had already been developed for estimation of isoprenoid cytokinins (Badenoch-Jones et al. 1987a, Hocart et al. 1988). The addition of 2-^{3}H-labeled cytokinins of the highest activity available (approximately 1.0 TBq · mmol^{-1}) to the extracts facilitated detection of the immunoactive fractions, giving better resolution of compounds that elute close together, e.g., N^6-benzyladenine and isopentenyladenine, as well as a measure of the percentage recovery of each cytokinin after purification. Chromatographic procedures used do not generally resolve these cytokinins, and thus the immunoactivity attributed to isopentenyladenine and related derivatives could in some cases turn out to be due to the presence of N^6-benzyladenine-like substances (Nandi et al. 1989b). HPLC-ELISAs of extracts of teratoma shoot culture derived from transformed *Solanum* leaf discs by T-DNA gene 4 (*ipt*) indicated that the high endogenous levels of N^6-benzyladenine-type cytokinins found in crown galls (Nandi et al. 1989a) and teratoma shoot culture (present study) are probably induced by elevated levels of isoprenoid cytokinins (see Ondřej et al. 1990) rather than synthesized by enzymes encoded in T-DNA. T-DNA gene 4 involved in the production of isoprenoid cytokinins may well interfere with wild-type biosynthetic pathways of aromatic cytokinins. Furthermore, it has been postulated that cytokinins induce their own synthesis when present at higher than threshold concentration (Meins and Hensen 1985). The effects of exogenous cytokinins on the accumulation of endogenous zeatin and zeatin riboside which would support this hypothesis have been reported (Mok et al. 1982, Vaňková et al. 1987). Thus, the data obtained in the present study indicate that an increase in isoprenoid cytokinin levels due to transgenesis by T-DNA gene 4 can induce an increase in the level of aromatic cytokinins in plant cells. The identity of

the aromatic cytokinins of potato transformants is under investigation.

The presence of putative N^6-(*meta*-hydroxybenzyl)adenine cytokinins in *P.* × *Robusta* leaves was confirmed by gas chromatography-mass spectrometry (Strnad et al. 1997), but the identification of endogenous N^6-benzyladenine in this tissue is still in progress. The unambiguous identification of aromatic cytokinins and their detection at appreciable levels in mature poplar leaves, fruits of *Zantedeschia aethiopica* (Chaves das Neves and Pais 1980), an old anise cell culture (Ernst et al. 1983b), and crown galls (Nandi et al. 1989b) clearly showed that these cytokinins are present in cells that have ceased cell division. Because of this they have been regarded as senescence-retarding factors (Horgan et al. 1975). The relatively low level of N^6-benzyladenosine and its hydroxylated derivatives in growing *P.* × *Robusta* leaves is at least consistent with this idea.

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