Development of a Plant Growth Regulator, ACC

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ACC is a naturally occurring non-protein amino acid that is rapidly converted to ethylene in plant tissues. ACC has unique activity as a chemical thinning agent in apples and stone fruits that provides fruit growers with a new tool to reduce their dependence on labor for crop load management. Furthermore, ACC is an alternative to ethephon in programs for coloring table grapes. ACC is highly safe for humans, animals and the environment, and is exempt from tolerance with the US EPA.

Introduction

The gaseous compound ethylene was first reported to control plant growth more than a century ago[1) – 3)] and is now considered one of the five "classical" plant hormones along with auxins, gibberellins, cytokinins and abscisic acid. Ethylene regulates various developmental processes in plants including seed germination, ripening, senescence, organ abscission, pigment formation and sex determination. Given the profound effects of ethylene on various aspects of plant development it has long been used in commercial fruit production systems globally. One of the main uses of plant growth regulators in apple production is for chemical thinning i.e. triggering the abscission of flowers and fruits. Chemical thinning is an alternative to using expensive hand labor to reduce the number of fruit on each tree and therefore increase fruit size and value at harvest. However, since there are no effective chemical options for thinning in stone fruit such as peaches, nectarines and sweet cherries then the reduction in fruit number per tree is exclusively achieved using increasingly expensive manual labor (**Fig. 1**).

The ethylene releasing agent 2-chloroethyl phosphonic acid (ethephon) has been used as a thinning agent in apples and to promote red color (anthocyanin) formation in table grapes. There is significant risk of overthinning apples with ethephon particularly if temperatures are high around the time of application[4)]. Due to the unpredictable thinning response to ethephon it is commonly used as a last resort in apple thinning programs. Ethephon is not used for chemical thinning in stone fruit orchards because it can trigger a physiological response called gummosis that can lead to declining tree health and ultimately death. Furthermore, ethephon has been coming under increasing regulatory scrutiny due to MRL issues when used close to harvest e.g. for color formation in crops such as table grapes and blueberries. There was a need

Fig. 1 Importance of manual fruit thinning for enhancing harvest value in stone fruit orchards

Stone fruit orchards use expensive manual labor to reduce the number of fruit per tree early in the season in order to achieve larger fruit and higher crop value at harvest. The photograph on the left above shows an individual peach fruit with a high number of fruit per shoot whereas the photograph on the right represents the commercial crop load (number of fruit per shoot) after hand thinning.

therefore to find an alternative fruit abscission/coloring agent with thinning activity in stone fruit that did not leave residues.

1-Aminocyclopropane-1-carboxylic acid (ACC, **Fig. 2**) was identified as the precursor of ethylene in plants in 1979[5] and as such could be considered a candidate agricultural compound using the plants own enzyme systems for the final conversion to ethylene. Conversion of ACC to ethylene is typically rapid in apples[6] and stonefruit[7] following ACC application, peaking one day after application and gradually declining to background levels by eight days. It should be noted that the breakdown of ethephon into ethylene in plants is a strictly physico-chemical reaction that is promoted under the alkaline pH conditions within the cytoplasm of the cell[8] and is therefore likely to have very different thermal kinetics compared to the enzymatic conversion of ACC to ethylene. Since the mechanisms whereby ethephon and ACC are converted to ethylene follow very different reaction pathways it was logical to hypothesize that they may also have different efficacy characteristics when used for thinning fruit crops such as apples and stone fruit or for coloring table grapes.

In this paper, we report on the biological effects, formulation, manufacturing process, mode of action, and safety for mammals and the environment.

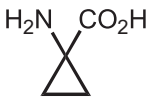


Fig. 2 Chemical structure of ACC

Biological Effects

1. Apple thinning

An apple thinning trial was conducted on the cultivar 'Modi' in Argenta in the Emilia-Romagna region of Italy in 2020. ACC was applied as the Accede 40% water soluble granule (SG) formulation to four tree plots at either 100, 200, 300 or 600 mg/L and compared to an untreated control. The highest concentration was included to test for crop safety according to EPPO standard PP 1/135 (4). The treatments were applied with a motorized backpack handgun in a spray volume of 1,000 liters per hectare when the mean fruit diameter was 18 mm. The study was arranged in a randomized complete block design with four replications. On the center two trees in each plot the total number of flowers and fruit per tree was counted at bloom and immediately prior to hand thinning, respectively. The thinning efficacy of ACC was quantified as fruit set (the number of fruit per 100 flower clusters). All of the trees in the study were hand thinned to a commercial crop load 48 days after ACC application according to standard crop load management practices (approx.110 fruit remaining on each tree) and the number of fruit removed from each tree by hand thinning was recorded. Mean fruit weight and yield were recorded at harvest. These data are presented in **Fig. 3**.

There was a linear reduction in fruit set (**Fig. 3.i**) and the number of fruit needing to be removed per tree by hand thinning in order to reach a commercial crop load (**Fig 3.ii**) in response to increasing rates of ACC. Furthermore, increasing rates of ACC resulted in a linear increase in both mean fruit weight at harvest

(**Fig 3.iii**) and fruit yield per hectare (**Fig 3.iv**). These data provide a clear indication of the value proposition that can result from use of ACC as an apple thinner. In this instance the grower would require less labor for hand thinning and the crop value is increased as the result of an increase in both mean fruit weight and fruit yield per hectare. Even though all of the trees had a similar number of fruit per tree after hand thinning to a commercial crop load, the increase in yield resulting from ACC treatments is a consequence of the much earlier reduction in fruit number leading to higher mean fruit weight.

ACC is registered in the US, Mexico, Canada, Brazil, South Africa and Chile for use as an apple thinning agent with registration pending in several other countries. ACC can be applied in a thinning program along with existing thinning agents such as carbaryl, naphthalene acetic acid and 6-benzyladenine at rates from 200 mg/L to 400 mg/L. ACC can be applied to apples during the period from full bloom until the average diameter of the king fruitlets is 25 mm. Apple fruitlets are most sensitive to ACC when they are 15 – 20 mm in diameter whereas most other commercial chemical thinning agents are effective earlier, when fruitlets are 8 – 12 mm in diameter. This unique feature of ACC makes it a promising tool for apple growers in seasons when climatic conditions during the early thinning window are not suitable or when the response to fruitlet thinning sprays during the earlier application window is insufficient.

2. Stone fruit thinning

A thinning trial was conducted on the nectarine cultivar 'Orion' in Giannitsa in the Pella region of Greece in 2020. ACC was applied as the Accede SG formulation to single tree plots at either 100, 200, 300, 400, 500, 800 or 1,000 mg/L and compared to an untreated control without hand thinning and a hand thinned control that did not receive ACC application (Hand thinned). The higher concentrations (800 mg/L and 1,000 mg/L) were included to test for crop safety according to EPPO standard PP 1/135 (4). The treatments were applied with a motorized backpack sprayer in a spray volume of 800 liters per hectare at the pink bud stage of development (BBCH 57). The study was arranged in a randomized complete block design with six replications. The thinning efficacy of ACC was quantified as fruit set (the number of fruit per m shoot length prior to hand thinning) on 15 to 20 sample limbs per tree that provided an average of 350 flowers in total per tree just prior to hand thinning (48 days after application). After thinning efficacy data

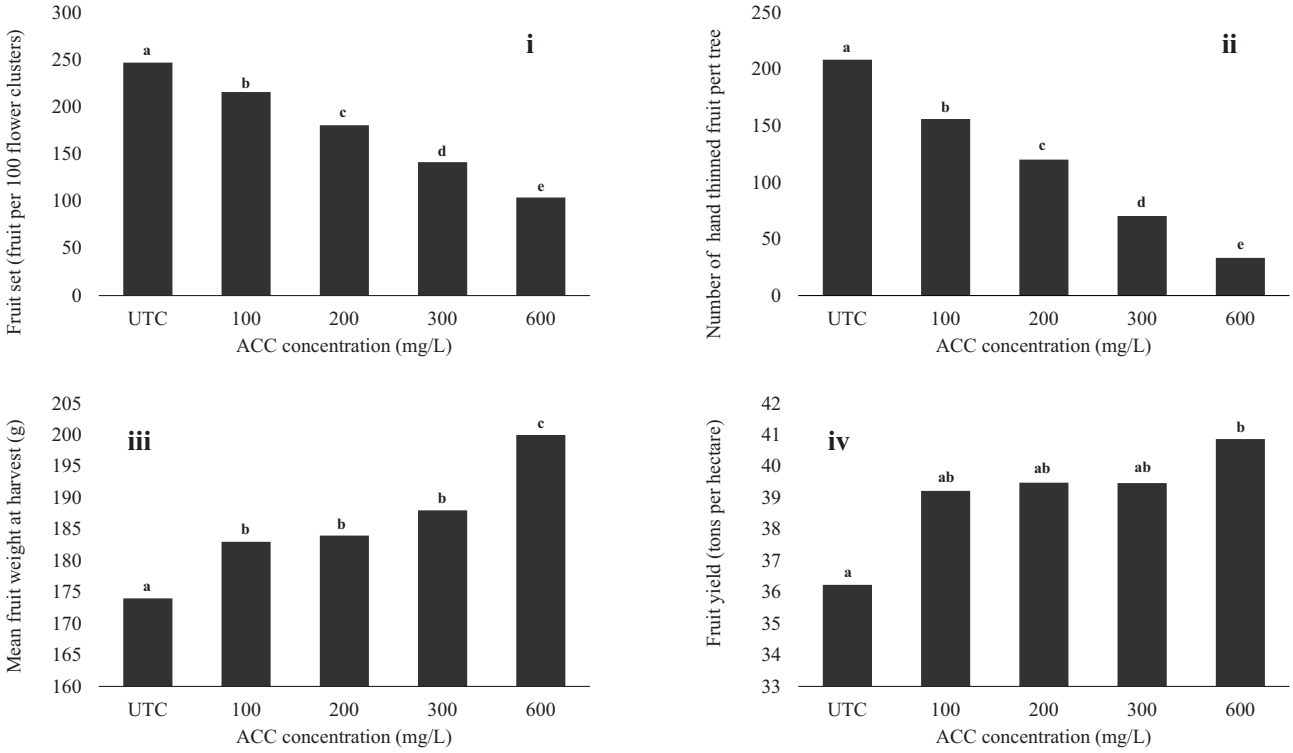


Fig. 3 Effect of ACC concentration on fruit set (i), number of fruit removed by hand thinning to reach a commercial crop load (ii), mean fruit weight at harvest (iii) and fruit yield per hectare (iv) of apple cv.'Modi'
Bars with different letters denote significant differences at $P<0.05$ using Tukey's HSD test.
UTC, untreated control

were collected all of the trees were hand thinned to a commercial crop load according to standard crop load management practices and the number of fruit removed from each tree by hand thinning counted. Mean fruit weight and yield of fruit per tree was recorded at harvest. These data are presented in **Fig. 4**.

There was a significant linear increase in thinning activity with increasing concentrations of ACC above 300 mg/L as measured by the reduction in fruit number per meter of shoot length (**Fig. 4.i**). There was a parallel reduction in the number of fruit removed from each tree by hand thinning as a consequence of the thinning activity of ACC (**Fig. 4.ii**). In this study concentrations of ACC above 400 mg/L tended to over-thin as determined by the reduction in yield (**Fig. 4.iii**) compared to the hand thinned treatment. In this instance the over thinning that occurred resulted in significantly larger fruit at harvest (**Fig. 4.iv**), however the increase in mean fruit weight did not compensate for the reduced number of fruit per tree so that yield was reduced (**Fig. 4.iii**). These data demonstrate that ACC can reduce fruit set of nectarines, resulting in a significant reduction in the requirement for hand thinning and an increase in fruit weight at harvest. However, they also show that higher concentrations of ACC can result in over thinning and a reduction in fruit yield at harvest.

In our efficacy studies we have found there can be significant differences in the sensitivity of stone fruit cultivars to ACC. For this reason, it is important to provide cultivar-specific recommendations to the grower to ensure that a suitable concentration of ACC is applied that will mitigate the risk of over or under thinning. Furthermore, the goal for use of ACC as a crop load management tool in stonefruit production is not to completely eliminate the need for hand thinning but rather to reduce fruit set, and therefore the need for hand thinning, by 40 to 60 percent.

ACC is registered for stone fruit thinning in the US, Chile and South Africa. ACC can be applied to crops including peaches, nectarines, plums and sweet cherries from the pink bud stage (BBCH 57) until petal fall (BBCH 67). ACC should not be applied if there is a risk of frost during bloom. If frost occurs at this time

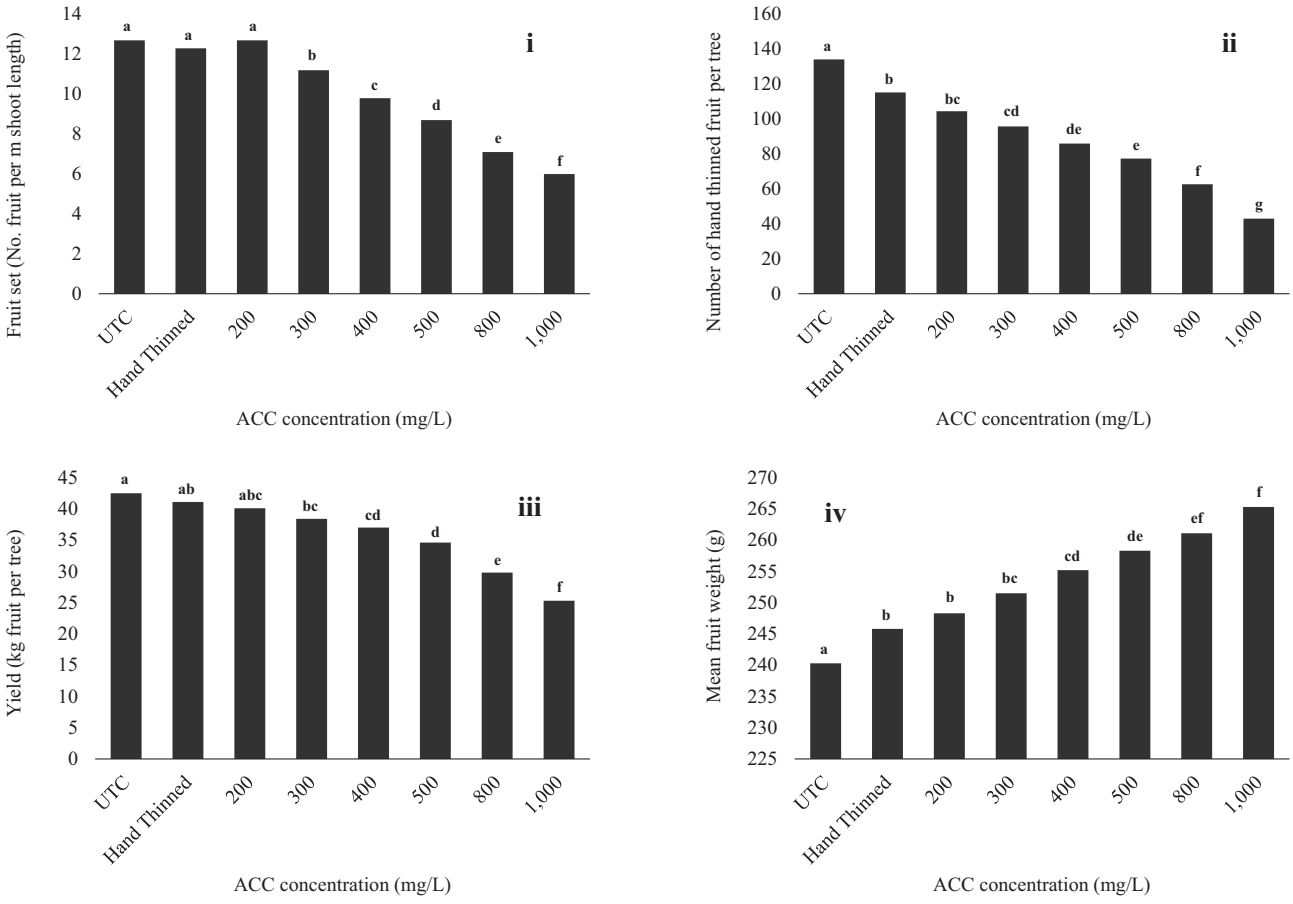


Fig. 4 Effect of ACC concentration on fruit set (i), number of fruit removed by hand thinning to reach a commercial crop load (ii), fruit yield per tree (iii) and mean fruit weight at harvest (iv) of nectarine cv. 'Orion'
Bars with different letters denote significant differences at $P<0.05$ using Tukey's HSD test.
UTC, untreated control

Fig. 5 Effect of ACC concentration on coloration of 'Red Globe' table grapes
Photographs were taken one week after the first ACC application.
UTC, untreated control

growers are advised to wait until damage to flowers and buds can be assessed before determining if application of ACC is needed for additional thinning. ACC cannot be applied to peaches or nectarines after petal fall due to an increased risk of leaf yellowing and drop.

3. Grape coloration

The effects of ACC concentration on color development of 'Red Globe' table grapes was evaluated in a study in Gran Chimú, Peru. ACC was applied as the Accede 10 % soluble liquid (SL) formulation to three-vine plots in a water volume of 1,000 liters per hectare. The treatments were an untreated control and ACC at 112, 224, 336 or 448 mg/L (corresponding to 1, 2, 3 and 4 L of formulated product per hectare). The experiment was arranged as a randomized complete block design with four replications. ACC treatments were applied when 80 – 100 percent of the berries reached the berry softening stage (véraison) and a second application was made seven days later. Forty individual bunches were tagged on the central vine within each plot for color assessment. The vines were harvested twice, only removing bunches that had reached a commercially acceptable level of color development at each harvest date. The harvests were made 21 days and 28 days after the first ACC application. The weight of bunches removed at each harvest date was expressed as a percentage of the total weight.

Red color development due to accumulation of anthocyanin pigment in the berry skin can develop relatively quickly following ACC application when the environmental conditions are ideal. In this study on 'Red Globe' a clear dose-dependent effect of ACC concentration on color development could be observed as early as seven days after the first application (**Fig. 5**). The stimulation of red color development by ACC was evident at the first harvest when only 37 percent of the bunches on control vines had reached a commercially acceptable level of red color development compared to 80 percent of bunches reaching acceptable red color for the highest concentration of ACC (**Fig. 6**).

The responses in this study demonstrate that use of ACC for coloration of table grapes can result in harvest of more bunches at early harvests, when prices received in the market may be higher. Other studies we have conducted have shown that ACC may reduce the number of times that a grower needs to harvest the crop and may also reduce the number of bunches that are unmarketable due to poor color development at the final harvest.

4. Temperature effects on the conversion of ACC to ethylene

The effect of temperature on ACC-induced ethylene production in leaves from peach and apple trees was investigated through a series of controlled environment studies. Ethephon was included in the experiments at an equimolar concentration for comparison. 'Flavorich' peach trees and 'Geneva 41' apple rootstocks were grown in a temperature-controlled greenhouse. When the trees and rootstocks developed enough leaves, they were sprayed to drip with 300 mg/L ACC or 429 mg/

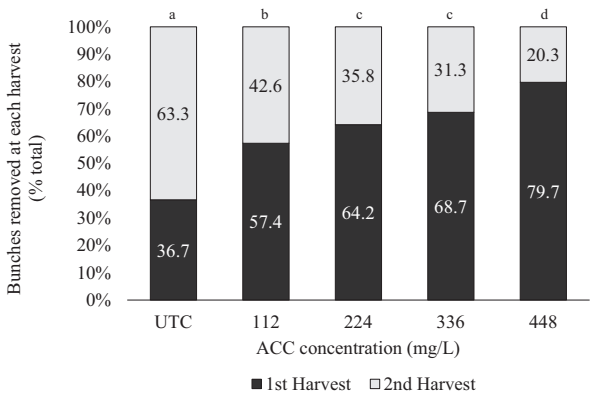


Fig. 6 Effect of ACC concentration on the percentage of bunches removed at each harvest
Only bunches that had reached the commercially acceptable level for red color were removed at each harvest.
Bars with different letters denote significant differences at $P < 0.05$ using Fisher's LSD test. Percentage data were arcsine square root transformed prior to analysis.
UTC, untreated control

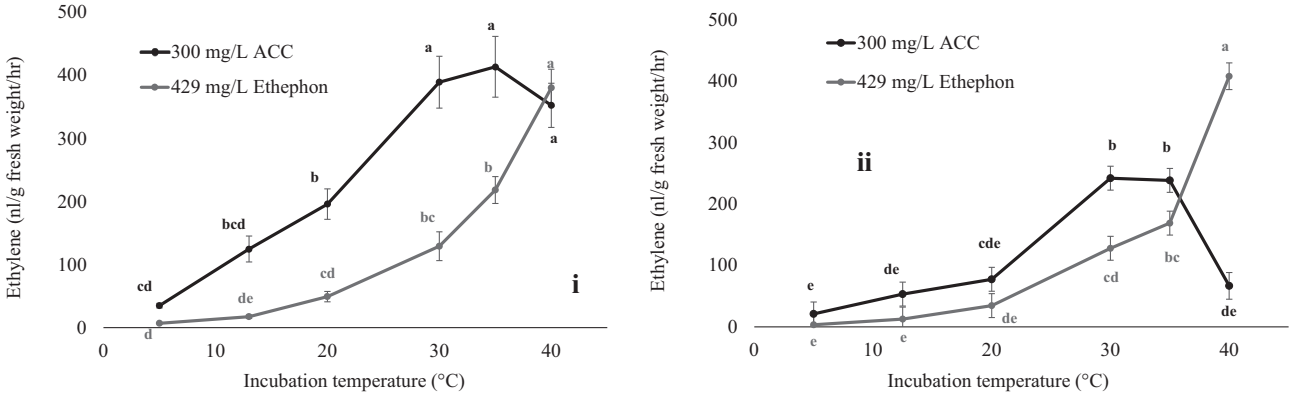


Fig. 7 Temperature-dependency of ACC- or ethephon-induced ethylene emission in 'Flavorich' (i) and 'Geneva 41' apple rootstock (ii) leaves
Data points with different letters denote significant differences at $P<0.05$ using Tukey's HSD test.
Error bars represent ± SE (n = 5).

Table 1 Physical properties of Accede SL and Accede SG formulations

Physical property	Accede SL	Accede SG
Active wt/wt%	10%	40%
Formulation Type	Soluble Liquid (SL)	Soluble Granule (SG)
Color	Clear yellow	Off-White Granule
pH (1% w/v in water)	5.7	5.2
Density (g/ml)	1.12	0.4

L ethephon and returned to the greenhouse overnight. The following day leaves were excised from the trees, weighed, sealed in jars, and incubated in the dark at temperatures between 5 and 40 °C. Headspace ethylene was analyzed via gas chromatography with flame ionization detection.

Ethylene emission was temperature dependent for both the ACC and ethephon treatments (**Fig. 7**). The effects of temperature on ethylene formation exhibited a similar trend for both peach and apple leaves. ACC-induced ethylene emission increased linearly between 5 and 20 °C and was consistently higher than ethephon-induced ethylene emission in this temperate range. ACC-induced ethylene emission plateaued between 30 °C and 35 °C, then declined thereafter, while ethephon-induced ethylene emission continued to increase up to 40 °C. These findings support previous research demonstrating different kinetics for ethylene emission for these two plant growth regulators. Furthermore, the results show that ACC releases lower amounts of ethylene compared to ethephon at high temperatures. This difference can reduce the risk of over-thinning when temperatures are high following application.

Formulation

ACC is formulated into a liquid concentrated formulation, Accede SL, at 10% wt/wt active and a soluble extruded granular formulation, Accede SG, with 40% wt/wt active. The physical properties of these two formulations are listed in **Table 1**.

Formulation of Accede SG involves blending of the active ingredient with inerts, binder and water to form a dough that is suitable for extrusion. Initial pilot scale production runs presented significant heat build-up during dough preparation which restricted optimal binder addition, resulting in poor attrition resistance of the granules. These issues were traced to ACC's uniquely strong affinity to water. In contrast to other plant growth regulator active substances such as gibberellic acid and S-abscisic acid, ACC and water were found to produce previously unknown hydrated forms, generating heat in the process. This heat altered the physical properties of dough, impeding optimal water addition and triggering processing variability. Multiple process improvements were implemented at the production level to successfully address these scale-up challenges.

Fig. 8 Appearance of Accede SG formulation

Both formulations yield slightly acidic pH in water and are not expected to cause any issue during field applications. The liquid formulation can be mixed completely and instantly into spray water even under cold conditions. The granule formulation disperses readily into water and dissolves completely after a few inversions. The appearance of the SG formulation is shown in **Fig. 8** and is nearly dust-free. Both formulations have excellent chemical stability and are compatible with common pesticides that are used in commercial practice during the time of typical applications.

Manufacturing process

Since the 1980s, many companies have shown interest in ACC, and numerous studies have been conducted on its production method. Although various synthetic routes have been proposed, isolating ACC in its free form has presented technical challenges. ACC is a non-protein amino acid with a low molecular weight of 101.1 and is highly soluble in water. However, the byproduct salts generated during the isolation process of its free form also exhibit high water solubility and possess very similar physical and chemical properties to ACC, making their separation and purification difficult (**Fig. 9**).

Through extensive investigation, it was found that by selecting the appropriate reagents and solvents for neutralization and precisely controlling the crystal form, the solubility difference between ACC and the byproduct salts could be increased, allowing for the isolation of ACC in its free form. Furthermore, selecting the optimal production route and advancing research into the manufacturing process led to the establishment of a high-quality biorational product manufacturing method.

Toxicity, metabolism, and residues

1. Mammalian toxicity

(1) Acute toxicity, irritation, and skin sensitization

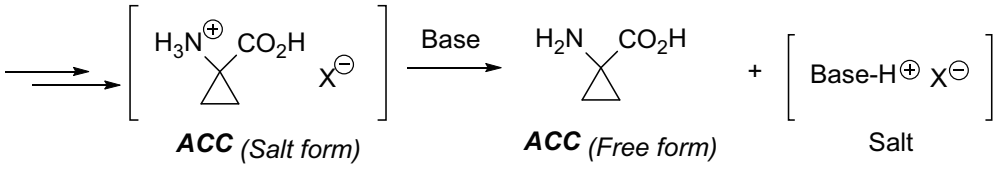
The acute toxicity of ACC technical grade active ingredient (TGAI) is extremely low, with the median lethal dose (LD_{50}) values exceeding 5,000 mg/kg body weight in rats for oral administration and dermal application, and the median lethal concentration (LC_{50}) value exceeding 5,100 mg/m^3 in rats for inhalation exposure. No deaths or toxic signs were observed following any administration route. The acute toxicity of Accede SG is also extremely low, with no deaths or toxic signs observed following oral administration or dermal application at 5,000 mg/kg body weight or inhalation exposure at 5,100 mg/m^3. The eye and skin irritation potential of ACC TGAI is minimal and slight, respectively. For Accede SG, no eye or skin irritation was observed. Neither the TGAI nor the SG formulations of ACC caused skin sensitization (**Table 2**).

(2) Subacute and chronic toxicity and carcinogenicity

In subchronic, chronic toxicity, and carcinogenicity studies conducted using rats and mice, repeated administration of ACC TGAI resulted in lower body weight, but no other toxic effects were observed. Additionally, no carcinogenicity was observed in rats (**Table 3**).

(3) Developmental and reproductive toxicity

In the developmental toxicity (teratogenicity) studies using rats and rabbits, no teratogenicity was observed



ACC *(Salt form)* ***ACC*** *(Free form)*

Fig. 9 Isolation method of ACC (free form)

in fetuses. In an extended one-generation reproductive toxicity study using rats, no effects on reproductive or nursing behavior were observed (**Table 4**). Although vacuolation in the brain was observed in the ACC TGAI administration groups, subsequent studies of mechanism indicated that no vacuoles formed during the animals' lifetime but rather vacuolation resulted from immersing the brain in organ fixation

Table 2 Acute toxicity summary of ACC

Test type	ACC TGAI	Accede SG
Rat acute Oral (LD_{50})	> 5,000 mg/kg	> 5,000 mg/kg
Rat acute Dermal (LD_{50})	> 5,000 mg/kg	> 5,000 mg/kg
Rat acute inhalation (LC_{50})	> 5,100 mg/m^3	> 5,100 mg/m^3
Eye irritation (Rabbit)	Minimally irritating	Non-irritant
Skin irritation (Rabbit)	Slightly irritating	Non-irritant
Skin Sensitization (mouse)	Non-sensitizer	Non-sensitizer

TGAI, technical grade active ingredient
SG, water soluble granule

Table 3 Subacute and chronic toxicity summary of ACC

Species	Administration route and duration	Dose	NOAEL (mg/kg/d)
Rat	Dermal, 28 days	421, 738, 1,054 mg/kg/d	Male: 1,054 Female: 1,054
Rat	Oral (Dietary), 28 days	5,00, 8,700, 11,600 ppm	Male: 1,185 (11,600 ppm) Female: 1,245 (11,600 ppm)
Rat	Oral (Dietary), 13 weeks	750, 3,000, 12,000 ppm	Male: 794 (12,000 ppm) Female: 963 (12,000 ppm)
Rat	Oral (Dietary), 24 months	1,000, 3,000, 10,000 ppm	Male: 393 (10,000 ppm) Female: 138 (3,000 ppm) No carcinogenicity
Mouse	Oral (Dietary), 13 weeks	1,500, 3,500, 7,000 ppm	Male: 883 (7,000 ppm) Female: 1,071 (7,000 ppm)

NOAEL, no-observed-adverse-effect level

Table 4 Developmental and reproductive toxicity summary of ACC

Study	Species	Administration route and duration	Dose	NOAEL (mg/kg/d)	
Developmental toxicity	Rat	Oral (Dietary) Days 6–21 of gestation	5,000, 8,000, 13,000 ppm	Maternal Fetal	982 (13,000 ppm) 982 (13,000 ppm)
	Rabbit	Oral (Gavage) Days 7–28 of gestation	125, 250, 500 mg/kg/day	Maternal Fetal	250 500
Extended one generation reproductive toxicity	Rat	Oral (Dietary)	2,500, 5,000, 10,000 ppm	Parental	Male: 271 (5,000 ppm) Female: 647 (10,000 ppm)
				Offspring	Male: 543 (10,000 ppm) Female: 647 (10,000 ppm)
				Reproductive	Male: 701 (10,000 ppm) Female: 763 (10,000 ppm)

NOAEL, no-observed-adverse-effect level

solution (10% neutral buffered formalin) post-mortem, suggesting that this was not an adverse effect.

(4) Neurotoxicity

No neurotoxic effects were observed in a 28-day repeated neurotoxicity study using rats (**Table 5**).

(5) Mutagenicity

In the reverse mutation study using *Salmonella typhimurium* and *Escherichia coli*, the gene mutation study using L5178Y mouse lymphoma cells, the chromosomal aberration study using Chinese hamster ovary-derived CHO-WBL cell lines, the *in vitro* micronucleus study using human peripheral blood lymphocytes, and the *in vivo* micronucleus study using mice, all results were negative. (**Table 6**).

2. Animal and plant metabolism

(1) Metabolism in animals

Following oral administration of ^{14}C-labeled ACC to rats, ACC was rapidly absorbed and distributed throughout the body. The absorbed ACC was excreted mostly unchanged in the urine within 24 hours without being metabolized. The absorption rate is estimated to be over 82%, with no residual or accumulation in the tissues.

(2) Metabolism in plants

Regarding the metabolism of ACC in plants, there are studies and literature evidence conducted on multiple crop groups such as fruit vegetables, leafy vegetables, legumes, cereals and root vegetables. Based on the information from these studies, the primary metabolic pathways of ACC are considered to be the production of ethylene via oxidative opening of the three-membered ring and the formation of MACC (malonyl-1-aminocylopropane-1-carboxylic acid) through malonic acid conjugation.

3. Environmental behavior and residues

(1) Degradation in water

In the hydrolysis and aqueous photolysis studies, ^{14}C-labeled ACC was stable in buffer solutions. On the other hand, in the aerobic mineralization study, ACC was rapidly degraded in natural water with a degradation half-life of 0.52 to 0.85 days. The major degradation pathway in water was considered to be the formation of 2-ketobutylic acid (2-KBA) through

Table 5 Neurotoxicity summary of ACC

Study	Species	Administration route and duration	Dose	NOAEL (mg/kg/d)
Neurotoxicity	Rat	Oral (Dietary), 28 days	2,500, 5,000, 10,000 ppm	Male: 755 (10,000 ppm) Female: 829 (10,000 ppm)

NOAEL, no-observed-adverse-effect level

Table 6 Genotoxicity summary of ACC

Study	Study design	Results
Bacterial reverse mutation study	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2 *uvrA* −/+S9 mix: 1.5–5000 μg/plate	Negative
Mammalian cell gene mutation study (TK)	Mouse lymphoma cells L5178Y TK$^{+/-}$ −/+S9 mix: 100–2,000 μg/mL	Negative
In vitro chromosomal aberration study	Chinese Hamster Ovary (CHO-WBL) Cells −/+S9 mix: 253–1,010 μg/mL	Negative
In vitro micronucleus study	Human peripheral blood Lymphocytes −/+S9 mix: 50–1,012 μg/mL	Negative
In vivo micronucleus study	CD-1 mice bone marrow Oral (gavage): 500, 1,000, 2,000 mg/kg	Negative

the cleavage of the three-membered ring, and finally mineralization to carbon dioxide.

(2) Metabolism in soil

In the aerobic metabolism study, the degradation half-life (20 °C) of ^{14}C-labeled ACC was 0.5 – 3.0 days. The major degradation pathway in soil was considered to be similar to that in water, involving the formation of 2-KBA through the cleavage of the three-membered ring, and finally mineralization to carbon dioxide.

(3) Mobility in soil

The adsorption coefficient ($K_{Foc(ads)}$) of ACC, corrected for organic carbon content and calculated using the Freundlich adsorption isotherm, ranged from 0.57 to 300.7, with geometric mean values of 7.0 for pH <7 and 260.4 for pH >7.

(4) Residue in crops

When Accede SG was applied to apples at 400 g a.i./ha in a single spray, the residual concentration of ACC was below the limit of quantification (<0.04 mg/kg). Furthermore, when Accede SG was applied to peaches and nectarines at 480 g a.i./ha in a single spray, the maximum residual concentrations of ACC at harvest maturity were 0.08 mg/kg and 0.07 mg/kg, respectively.

4. Effects on non-target organisms

Table 7 summarizes the test results for aquatic animals and plants, arthropods and birds.

(1) Effects on aquatic animals and plants

The acute toxicity values (LC_{50} / EC_{50} / ErC_{50}) of ACC TGAI for rainbow trout, *Daphnia magna* and freshwater green algae were >117, >105 and >0.21 mg/L, respectively. The toxicity values (ErC_{50}) of Accede SG for freshwater green algae was 0.21 mg/L. These values are sufficiently higher than the predicted concentration in environmental water expected from practical use, indicating that the effects on aquatic animals and plants are considered to be low.

(2) Effects on arthropods

The LD_{50} values of ACC TGAI for oral and contact administration to honeybees were >254.4 and >120.9 µg/bee, respectively. Moreover, LD50 values of Accede SG were >43.05 and >59.99 µg a.i./bee, respectively. The acute toxicity values (LR_{50}) of ACC in contact exposure tests for parasitic wasps (*Aphidius rhopalosiphi*) and predatory mites (*Typhlodromus pyri*) were >545 and 264 g/ha, respectively. These results indicate that ACC has a low effect on arthropods in practical use.

(3) Effects on birds

The acute toxicity of ACC TGAI to bobwhite quail was low, with an oral LD_{50} value of 343 mg/kg, indicating low effects on birds in practical use.

Table 7 Ecotoxicological summary of ACC on non-target organisms

Test substance	Test species		Test type	Results
ACC TGAI	Aquatic organisms	Rainbow trout	Acute (96 hours)	LC_{50} > 117 mg/L
		Daphnia magna	Acute (48 hours)	EC_{50} > 105 mg/L
		Green alga*1	Acute (72 hours)	ErC_{50} = 0.21 mg/L
	Arthropods	Honeybee*2	Acute oral (48 hours)	LD_{50} > 254.4 µg/bee
		Honeybee*2	Acute contact (48 hours)	LD_{50} > 120.9 µg/bee
	Bird	Bobwhite quail	Acute oral (14 days)	LD_{50} = 343 mg/kg
Accede SG	Aquatic organisms	Green alga*1	Acute (72 hours)	ErC_{50} = 0.21 mg/L
	Arthropods	Honeybee*2	Acute oral (48 hours)	LD_{50} > 43.05 mg/bee
		Honeybee*2	Acute contact (48 hours)	LD_{50} > 59.99 mg/bee
		Aphidius rhopalosiphi	Acute contact (2 days)	LR_{50} > 545 g/ha
		Typhlodromus pyri	Acute contact (7 days)	LR_{50} = 264 g/ha

*1 *Raphidocelis subcapitata*
*2 *Apis mellifera*
TGAI, technical grade active ingredient
SG, water soluble granule

Based on the above, the acute toxicity of ACC TGAI and Accede SG to mammals is extremely low and long-term exposure to ACC is unlikely to result in any risks to human health such as carcinogenicity, teratogenicity, or reproductive effects. Additionally, given the behavior of the compound in the environment and its effects on non-target organisms, ACC is considered to have no impact on the environment when used according to the method applied for registration.

Conclusion

ACC is a non-protein amino acid that is rapidly converted to ethylene *in planta* using the plants natural biochemical pathways. Ethylene regulates various developmental processes in plants including seed germination, ripening, senescence, organ abscission, pigment formation and sex determination. ACC is currently registered as a chemical fruit thinning agent in apple and stone fruit productions systems and for promoting red color formation in table grapes in various countries. ACC has unique activity as an apple thinning agent due to the greater sensitivity of fruit when they are 15-20 mm in diameter compared to existing apple thinning agents. ACC is the only registered thinning agent for stone fruit in several countries (as of October, 2025), providing growers with a chemical tool for crop load management that reduces their dependence on expensive labor for hand thinning. ACC can stimulate red color in table grapes without residue concerns, providing an advantage over the ethylene releasing agent ethephon. Given the diverse roles of ethylene in plant development there will be many opportunities to develop new uses for ACC in agricultural production systems in the future.

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