GRAY MOLD (BOTRYTIS CINEREA) CONTROL, YIELD AND QUALITY IN STRAWBERRY WITH VITAZYME BIOSTIMULANT AND ROVRAL FUNGICIDE

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SUMMARY. A small-plot, replicated experiment was conducted in Irapuato, Guanajuato, México, in order to study Gray Mold (*Botrytis cinerea.*) control, yield and quality in Fortuna strawberries (*Fragaria sp.*) by a natural formulation of brassinosteroids + triacontanol + beta-vitamins (Vitazyme), by the fungicide iprodione (Rovral WP 50), and by mixtures of both, in different dosages. Vitazyme, at 1 l ha^{-1} and 1.5 l ha^{-1}, and Rovral, at 1 and 1.5 kg ha^{-1} , in three fortnightly (15 day) foliar sprays, reduced significantly the incidence and severity of Gray Mold, and conversely increased significantly yields, incomes and net profits, both when applied alone, as well as when applied in combination in the same sprays and plots, and in the latter case (combined, at 1 l ha^{-1} + 1 kg ha^{-1} , respectively) showed the lowest incidences and severities of the disease and the highest yields, incomes, and net profits, i.e., their effects were additive or synergistic. Vitazyme also had a more persistent effect on the disease than Rovral over the three 5-day application-to-evaluation intervals. On the other hand, Vitazyme, in the rates of 1 l ha^{-1} and 1.5 l ha^{-1} showed significant and marked increases in quality parameters: brix or soluble solids percentage and thus juice sweetness, and in fruit firmness or consistency, and thus shelf-life, as well as a much higher yield of fruit harvested for packaging (better appearance and consequent higher price), while Rovral fungicide had no effect on any quality parameter in any dosage rate.

Keywords: *Botrytis cinerea;* strawberry ; Vitazyme; brassinosteroids; fungicide.

CONTROL DE MOHO GRIS (*BOTRYTIS CINEREA*), RENDIMIENTO Y CALIDAD EN FRESA CON BIOESTIMULANTE VITAZYME Y FUNGICIDA ROVRAL

RESUMEN. Se desarrolló un experimento de parcelas pequeñas replicadas, en Irapuato, Guanajuato, México, con el fin de estudiar el control de Moho Gris (*Botrytis cinerea*), rendimiento y calidad en fresa *(Fragaria sp.)*, var. Fortuna, por un formulado natural de brasinoesteroides + triacontanol + beta-vitaminas (Vitazyme), por el fungicida iprodiona (Rovral PH 50) y por la mezcla de ambos, a diferentes dosis. Vitazyme, a 1 l ha^{-1} y 1.5 l ha^{-1}, y Rovral, a 1 y 1.5 kg ha^{-1}, en tres aplicaciones foliares quincenales, redujeron significativamente la incidencia y la severidad de Moho Gris y, por el contrario, aumentaron significativamente los rendimientos e ingresos, tanto cuando aplicados solos, como cuando aplicados mezclados en las mismas aspersiones y parcelas, y en este último caso (mezclados, a 1 l ha^{-1} + 1 kg ha^{-1}, respectivamente) mostraron las menores incidencias y severidades de la enfermedad y los mayores rendimientos, ingresos y ganancias netas, por lo que sus efectos fueron aditivos o sinérgicos. Vitazyme tuvo efecto más persistente sobre la enfermedad que Rovral a lo largo de los tres intervalos de 5 días entre aplicación y evaluación. Por otro lado, Vitazyme, a dosis de 1 l ha^{-1} y 1.5 l ha^{-1} mostró aumentos significativos muy marcados de los parámetros de calidad: porcentaje de brix o sólido solubles (por tanto dulzura del jugo) y firmeza o consistencia del fruto, por tanto vida de anaquel, así como un mucho mayor rendimiento de frutas cosechadas para empaque (de mejor apariencia y consecuente mayor precio), mientras que el fungicida Rovral en ninguna dosis ni parámetro de calidad tuvo efecto.

INTRODUCTION

Gray Mold (*Botrytis cinerea* Pers.) is one of the most common pathogenic fungi, since it has the ability to infect more than 200 different plant host species, among them: tomato, onion and strawberry. It is one of the most destructive diseases in strawberry crop under field conditions (Zhang *et al*., 2007), causing serious economic losses, estimated around 30% of the total production and, between 40 and 50% in conditions of high humidity.

The use of fungicides is the main means for the control of Gray Mold in strawberry. Since strawberries bloom for a period of several months, it will require multiple applications throughout the cycle. *Botrytis* is becoming resistant to fungicides, due to its high genetic variability and the abundant production of spores. Strains of Gray Mold in strawberry, resistant to almost all fungicides with single-site modes of action, have been confirmed (Mercier, *et al*., 2010). Repeated application of the same fungicides increases selective pressure and fosters the development of resistance, so rotation and mixtures of fungicides with different modes of action are recommended.

Rovral 50 PH, **a**n iprodione-based **f**ungicide, with penetrating mode of action, locally systemic and translaminar, penetrates through the leaves and accumulates close to the site of penetration. It acts by affecting signal exchange of the membrane with the environment, lipid metabolism and cellular respiration, and interferes with DNA biosynthesis. It produces thickening and damage by membrane disruption. It belongs to the Hydantoin chemical group, an d is classified by FRAC as a monosite Dicarboximide fungicide, with intermediate to high risk of resistance.

In addition to defense with fungicides, the plant can express a systemic response to disease, which is called induced resistance (Van der Ent *et al*., 2009). One of the strategies studied in recent years has been the use of resistance-induction products in order to regulate populations of plant pathogens in crops. Induced resistance produces an increase in the expression of the natural defense mechanisms of plants against various types of pathogens (Zeller, 2006).

The resistance-inducing agents may be of two main types: chemical (Acibenzolar-S-methyl, β-aminebutyric acid, probenazol, saccharin, phosphite, biochar, etc.) and biological (mycorrhizal fungi, algae extracts, etc.) (Walters *et al*., 2013). It has been shown that, although using only disease control inducers or inductors, control is lower than that obtained with fungicides, the use of the combination of the two (with a reduced amount of fungicide) could provide higher levels of control of the disease. The idea is the application of the inducer at the beginning of the cycle, allowing less use of fungicide later (Walters *et al*., 2013). In addition to reducing the total cost and pollutant load, these products can help extend the durability of resistance in cultivars with genes for resistance to pathogenic strains (Romero *et al*.; 1998, Tally *et al*., 1999, quoted by Romero *et al*. 2001).

There is background and growing evidence that brassinosteroid hormones (BR) play an important role in the defense of the plant against agents of both biotic and abiotic stress (Bajguz and Hayat, 2009; Gomes, 2011; Vriet et al., 2012). The response of the plant to BR includes effects on signaling systems for defense against insects and fungi, in cell and stem elongation, cell division, vascular and reproductive development, membrane polarization, and proton pumping, the source/site of consumption ratios and stress modulation. Also their influence on gravitropism and on delay of leaf and fruit abscission has been reported (Clouse., *et al*, 1996).

The application of epibrassinolide (or epiBL) to 'Lux' barley heads reduced by 86% the severity of stem blight, caused by *Fusarium culmorum*, and reduced losses associated with the disease in grain weight by 33%. The growth of plants in soils treated with epiBL resulted in reduction of 28 and 35% in symptoms of seedling *Fusarium* blight, in 'Lux' and 'Akashinriki' barley, respectively. (Ali *et al*, 2013).

A more recent study, which sought a possible control strategy for citrus Huanglongbing (HLB) *Candidatus Liberibacter asiaticus*, applied a foliar spray of epibrassinolide (epiBL) to citrus plants infected with the causal agent of HLB. Bacterial estimations were reduced after treatment with epiBL under greenhouse and field conditions, but more markedly in the former. Known defense genes were induced in leaves by epiBL. With SuperSAGE technology, combined with next-generation sequencing, the induction of genes known to be associated with the defense response to bacteria and to the hormonal signal transduction pathways, were identified. Results showed that epiBL can provide a useful tool for HLB management (Canales *et al*. 2016).

Although none of the before quoted studies with BR were against *Botrytis*, or in strawberries or other berries, nor assessed BR mixed with fungicide, we expect that the use of BR in strawberry crop could help to achieve a strategy for the control of *Botrytis*.

Vitazyme it is a natural biostimulant, manufactured through a fermentation process from plant material, which has several active agents and modes of action. The active ingredients include: four brassinosteroids: homobrassinolide, dolicholide, homodolicholide and brassinone (22 ppm), the alcohol 1-triacontanol (130 ppm); and three B vitamins: B1 or thiamine (3.5 ppm) , B2 or riboflavin (0.2 ppm) and B6 or pyridoxine (1.5 ppm), which produce increases of chlorophyll content, of photosynthesis and of population of beneficial organisms in the rhizosphere, and thus, improvement of nutrition efficiency and resistance to various types of stress, resulting in greater growth, earlier and more abundant flowering and fruit-set, and higher crop yields and quality.

The objective of the present study was to evaluate the potential effect as resistance inducer for Gray Mold (*Botrytis cinerea* Pers.) control in mature strawberry fruits of a formulated mixture of brassinosteroid + triacontanol + B vitamins (Vitazyme), compared to the control by the commercial fungicide Rovral (iprodione), as well as by the combined application of both.

MATERIALS AND METHODS

The study was conducted on a commercial 'Fortuna' strawberry field, at 6 months from planting, under macro-tunnel conditions, with plastic mulch, located in La Mocha Farm, owned by Mr. Miguel Angel Montibeller Torres, km 3 Las Malvas Road, coordinates: 20 ° 40' 49.3 North and 101 ° 16' 48.8 East, Municipality of Irapuato, Guanajuato, Mexico.

Before the first application of both products, on January 24th, 2017, soil, leaf and ripe fruit samples were collected to verify the presence of *Botrytis sp* in the experimental site. Likewise, one day before the application of treatments, a purge of all fruits at physiological maturity was made in order to ensure that the fruits that would be harvested after applications, could show the effects of the evaluated products.

The trial was laid down in a completely randomized blocks design or layout, with 8 treatments or experimental variants (which are described in tables) and 6 repetitions, for a total of 48 experimental units or plots of 0.8 m width x 10 m length, an area per unit of 8 m^2.

Three foliar applications (sprays) of treatments were made, at fortnightly (fifteen day) intervals (1^{st}: January 30, 2017, 2^{nd}: February 13, 2017 and 3^{rd}: February 27, 2017), by means of a Pulmic Tropic backpack sprayer, attached to a hollow cone nozzle, and with 200 liters/ha application volume, equivalent to 1 liter for the 6 experimental units (48 m^2).

At 15 days after the last application (March 13, 2017) a strain of *Botrytis spp.*, at a rate of 100,000 spores per ml water, was inoculated to ensure the infection in all the experimental area.

Three evaluations were made: March 31, 2017 (1 month after last application), April 5, 2017 and April 10, 2017, at 46, 51 and 56 days after the second and mean of the three applications.

For the parameters of incidence and severity of disease, 5 ripe fruits were taken at random. Incidence was the number of fruits with any degree of visible damage, so each damaged fruit accounted for 20% of the total of 5 sampled. The percentage of *B. cinerea* damage was determined using a 5-grade scale, in which grade 0 relates to "healthy fruit (no visible damage)" and grades 1, 2, 3 and 4 relate to "up to 1%", "1 to 5%", "6 to 25%" and "26 to 50%" fruit damaged area, respectively. There were no fruits with more than 50% damage.

Once determined the level of damage per experimental unit, the *Townsend and Heuberger* formula was applied to determine the weighted mean Severity: $P = [\sum (n * v) / CM * N] * 100$, where P means Severity; n = # of fruits per each class on the scale; v = numeric value of each class; CM = highest category (always 4); N = total number of fruits of the sample (5).

For quality parameters of brix and firmness, ten samples were taken per treatment. Brix percentages were determined by placing one drop of juice from five crushed strawberries per sample in a Hanna digital refractometer, with 0 to 85% range, and 0.1 brix accuracy. Firmness was evaluated through the mechanical force required for the deformation of fruit tissues, right at the breaking point, expressed in Newtons (N), by means of a TA. XT2 texture analyzer, with calibration of 5 mm/s speed and 8 mm depth.

Yields were evaluated by weighing, disaggregated in "for packaging", "for process" and "total", fruits harvested in 3 pickings by 8 treatments, with a Bull King brand, model PCR-40, scale.

Analyses of variance of two factors: interval (days after application), with 3 intervals, and Vitazyme by Rovral treatment, with 8 treatments (also, in *Botrytis* Incidence and Severity, with 6 replications; and in Firmness and Brix, with 10 samples), and comparison of means by Tukey Test, in each of the above two factors, at level of significance of $\alpha = 0.05$, were made. Both types of analysis were applied to all parameters: Incidence and Severity of Botrytis, fruit Brix and Firmness, and yields per pickings.

Additionally, step by step, forward, multiple regression analysis were performed, at level of significance of $\alpha = 0.05$, of the *Botrytis* Incidence and Severity parameters.

RESULTS AND DISCUSSION

Incidence and Severity of *Botrytis*

Incidence and Severity of *Botrytis* by Analysis of Variance and Tukey Test

Table 1 shows the results of the analysis of variance and Tukey test of comparison of means, of two factors: factor 3 intervals of DAA x factor of 8 treatments of Vitazyme x Rovral (dosage rates between parentheses in Table 1), in variables Incidence and Severity of *Botrytis*.

The review of the above-quoted Vitazyme x Rovral treatments, showed several significant effects: (a) that all treatments that included Rovral or Vitazyme at the rates recommended by the manufacturers, of 1 and 1.5 kg ha^{-1} or l ha^{-1}, reduced *Botrytis* significantly; (b) when applied alone, Rovral (treatments 5 and 3) had greater reduction effect on the disease than Vitazyme (treatments 2 and 8); (c) the greatest disease reduction effects arose when both products Rovral and Vitazyme were applied in the same experimental units (treatments 6 and 4); and (d) there were no differences between the rates of Rovral of 1 and 1.5 kg ha^{-1} (Table 1, Figs. 1 & 2).

Table 1. Mean Botrytis Incidence and Severity by Vitazyme x Rovral treatments and interval (days) between application and assessment (DAA).

	Incidence	sig.*	Severity	sig.*
Intervals between application and evaluation				
46 DAA	41.4	b	18.5	b
51 DAA	48.8	ab	24.3	a
56 DAA	53.0	a	27.8	a
Standard error	2.912		1,458	
Vitazyme by Rovral treatments				
1 (V0, R0)	69.0	a	36.8	a
7 (V0.5, R0)	59.8	ab	31.6	ab
2 (V1, R0)	54.0	abc	28.2	abc
8 (V1.5, R0)	47.7	bc	21.5	bcd
5 (V0, R1)	43.3	bc	20.0	cd
3 (V0, R1.5)	40.2	bc	18.7	d
4 (V1, R1.5)	34.5	c	15.8	d
6 (V1, R1)	33.3	c	15.8	d
Standard error	1895		0.547	

* Means accompanied by a common letter do not differ significantly by Tukey ($\alpha = 0.05$).

Fig. 3 shows the percentages (considering the absolute control, Treatment 1, as 100% of the disease) of reduction of Gray Mold in strawberry with Vitazyme 1 l ha^{-1} alone, with Rovral 1 kg ha^{-1} alone and with the combined application of both (Treatments 2, 5 and 6, respectively, in Table 1). This Figure 3 highlights, in a simple way, the enhancer, booster or synergistic effect of Vitazyme on the effect of the fungicide. Note that this is not the maximum effects, but the mean effects in the three evaluations, which were best in the first evaluation and worst in the last evaluation (Figs. 4-5).

In terms of intervals (DAA) there was significant increase of the disease, both in incidence as in severity, at greater intervals (Table 1).

Fig. 1. Botrytis Incidence by Vitazyme x Rovral treatments (according to Table 1)

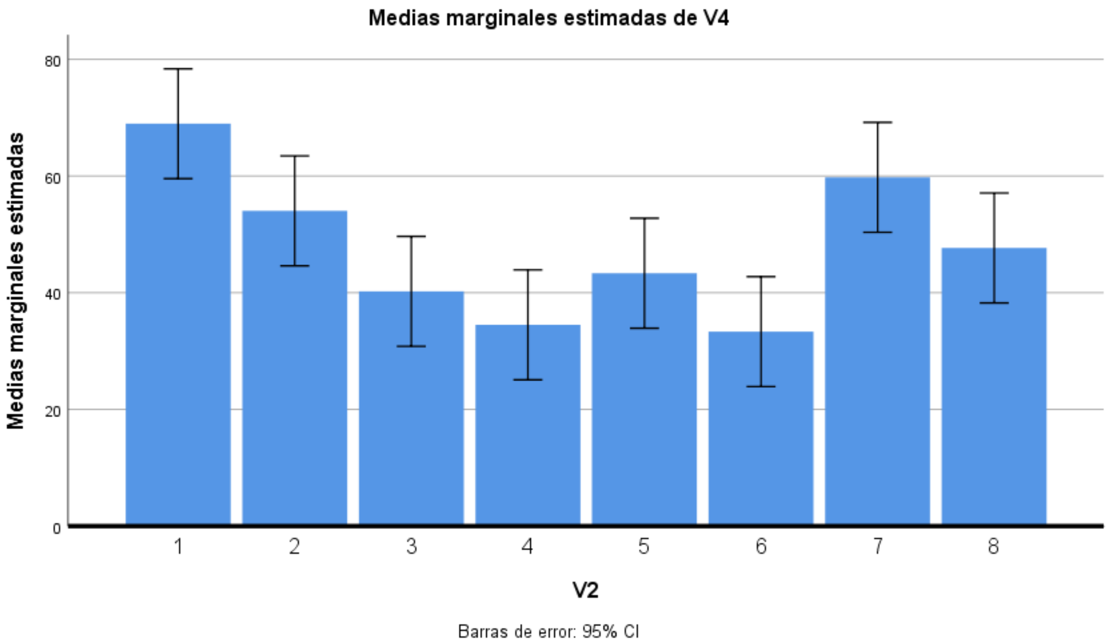


Fig. 2. Botrytis Severity by Rovral x Vitazyme treatments (according to Table 1)

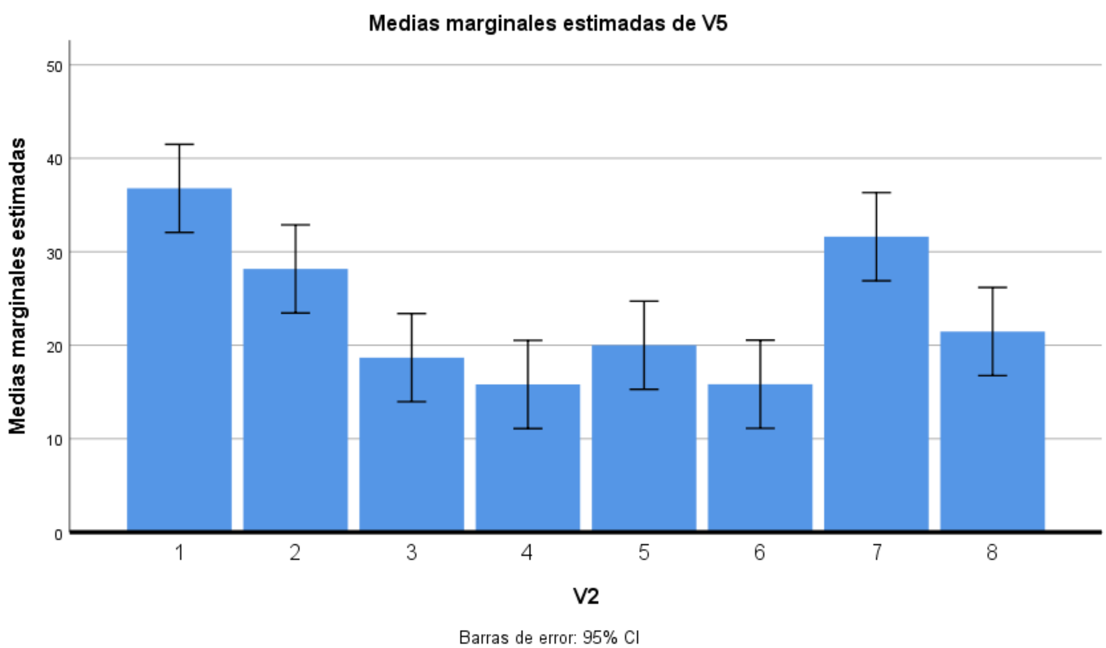
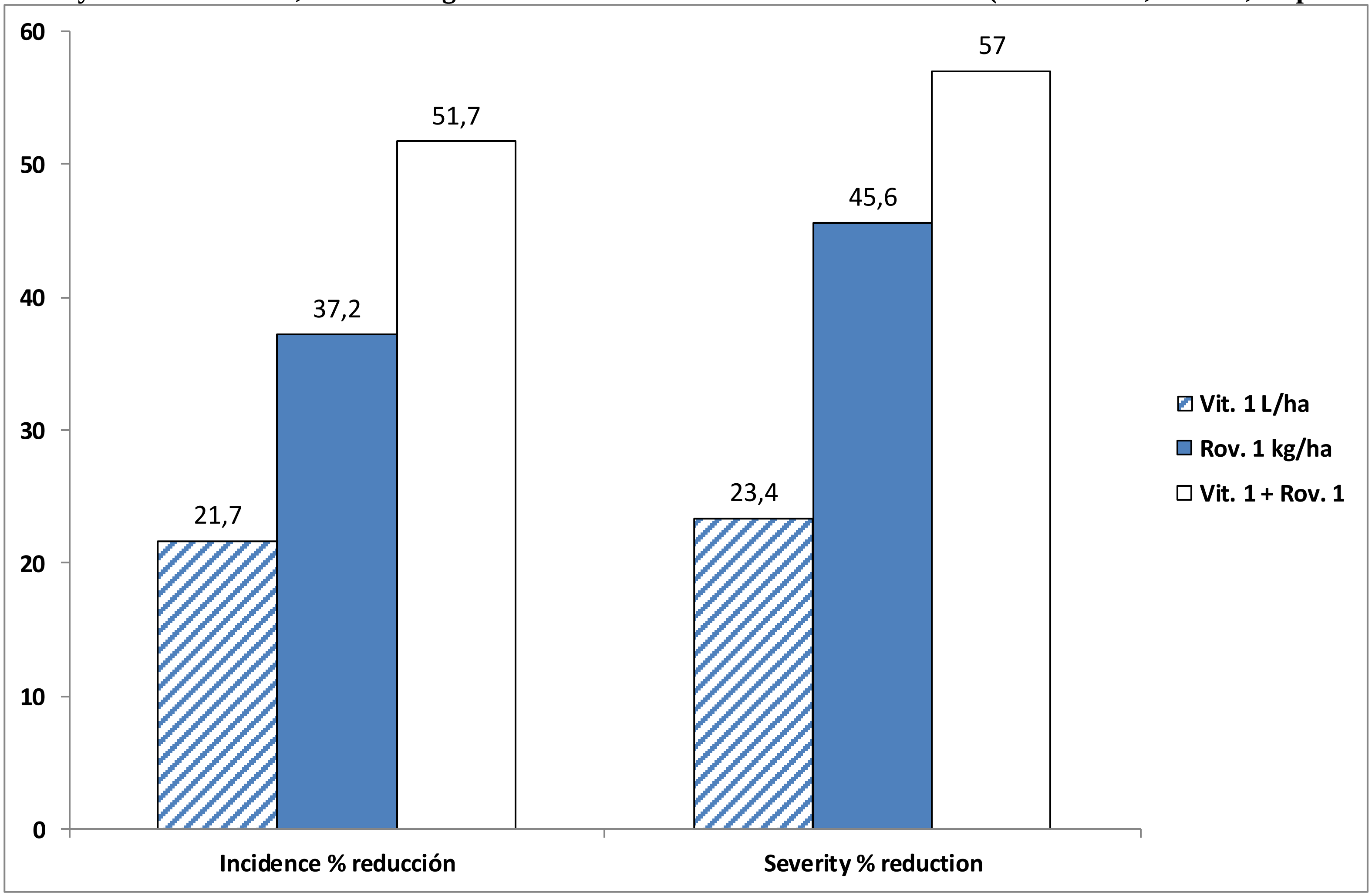


Fig. 3. Gray Mold reduction percentages (considering the control, Treatment 1 in Table 1, as 100% of disease) in strawberry with Vitazyme 1 l ha^{-1} alone, Rovral 1 kg ha^{-1} alone and with the combination of both (Treatment 2, 5 and 6, respectively).



Analysis of Incidence and Severity of *Botrytis* by Multiple Regression

The regression analyses confirmed the results of the analyses of variance, since they showed the same highly significant effects of Vitazyme and Rovral products on the disease, and supplemented, by analyzing the interactions between factors, fixed or independent variables (Interval, Vitazyme and Rovral). Ahead the statistical analyses by multiple regression and the estimated highly significant figures of Botrytis Incidence and Severity.

In the statistical analysis of multiple regression (linear, stepwise, forward), the final step (fourth step in Incidence and fifth in Severity) shows the best fit to the original data, which expresses the end result, when the statistical package SPSS finalized entering or removing terms, as required.

Both Rovral and Vitazyme had highly significant effects on the decrease in Incidence and Severity, which is the most important result. Although Rovral's effect (with no significant difference between 1 and 1.5 kg ha^{-1}) as an average was higher than that of Vitazyme (which showed progressive effects proportional to doses 0.5, 1 and 1.5 l ha^{-1}), both had significant effects and the best treatments were those where both were present.

The two products had NO interaction between them (the term Vit x Rov was excluded in the analysis of regression of both parameters), which means that the response to one did not change at various levels of the other, on the contrary, their effects were additive or synergistic when applied together, which could be seen, both by multiple regression and ANOVA.

On the other hand, the effect of Rovral (both Incidence and Severity) was influenced by the DAA or interval between application and evaluation, as you may see in the term DAA x Rovral, which was significant and included in the final regression models of both parameters, but not with Vitazyme (response not influenced by intervals), since in both parameters the term DAA x Vit was excluded from both final regressions. Thus, you may see in the regression figures of Incidence (Figs. 4 & 6), that in Rovral alone, the difference between 1.5 kg ha^{-1} and the absolute control (without Rovral and Vitazyme) varied from 35.3% in the initial evaluation at 46 DAA (66-30.7) to only 10.7% in the third evaluation at 56 DAA (66 - 55.3) (Fig. 6); and in Severity (Figs. 5 & 7) the difference between Rovral 1.5 kg ha^{-1} and the absolute control varied from 17.6% in the initial evaluation (31-13.4) to 11% on the third evaluation (37.6-26.6) (Fig. 7), i.e. very large variations between the maximum rate of Rovral and control from the first and the last assessments. However, with Vitazyme alone, the difference between 1.5 l ha^{-1} and the absolute control remained constant during the three assessments or intervals (DAA), which shows a more lasting effect on the disease in Vitazyme than in Rovral. Thus, in Vitazyme in the three evaluations such difference was, in Incidence of 16.8% (66-49.2) (Fig. 4) and in Severity of 12.4% (31-18.6; 34.3-21.9; and 37.6-25.2) (Fig. 5). Note (Figs. 4 and 5) that in the last assessment both parameters were slightly better (lower values) with Vitazyme 1.5 alone (Treatment 6, left to right) than in Rovral alone 1.5 (Treatment 6, left to right). The original or actual data show the same behavior described above for estimates of the interactions (DAA x Vitazyme non-significant and therefore more stable effect, while DAA x Rovral is significant and therefore shows less stable effect), in Incidence and Severity of Botrytis (Figs. 6-7).

Fig. 4. Incidence (%) of *Botrytis* by rates of Vitazyme and Rovral, according to model y=66.034 - 119.102 *Rov + 1.639*DAA*Rov - 11.240*Vit + 13 418*(Rov)^2. R=0.927; R^2=0.859; Std. Error Esti. = 5.93; ANOVA F=28.922, sig=0.000

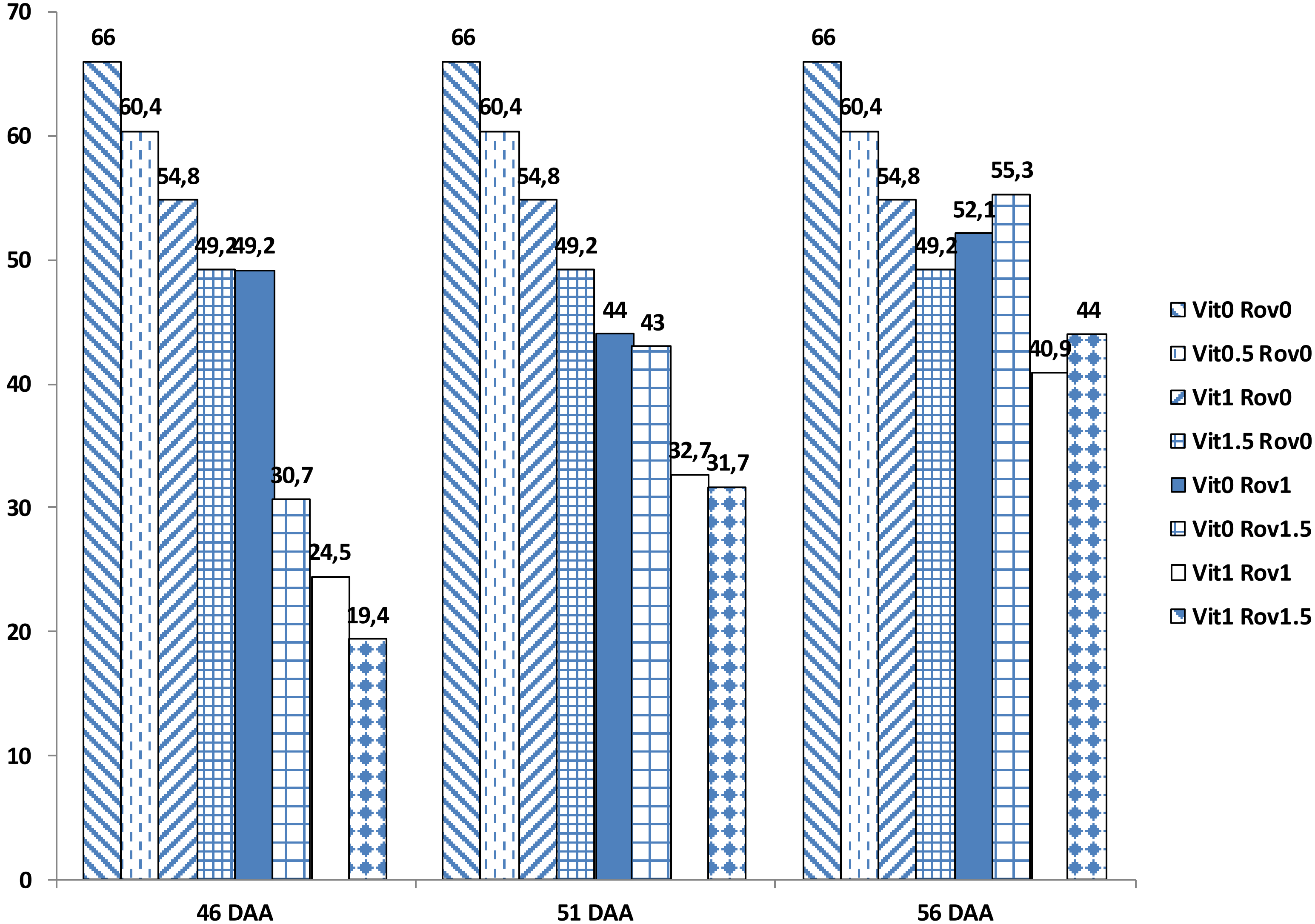


Fig. 5. Severity of *Botrytis* by rates of Vitazyme and Rovral, according to model $y = 0.813 - 44.102*Rov + 8.205*(Rov)^2 + 0.436*DAA*Rov - 5.519*(Vit)^2 + 0.657*DAA$. $R=0.973$; $R^2= 0.948$; Std. Error Estim. = 2.22; ANOVA $F=65.14$, sig = 0.000

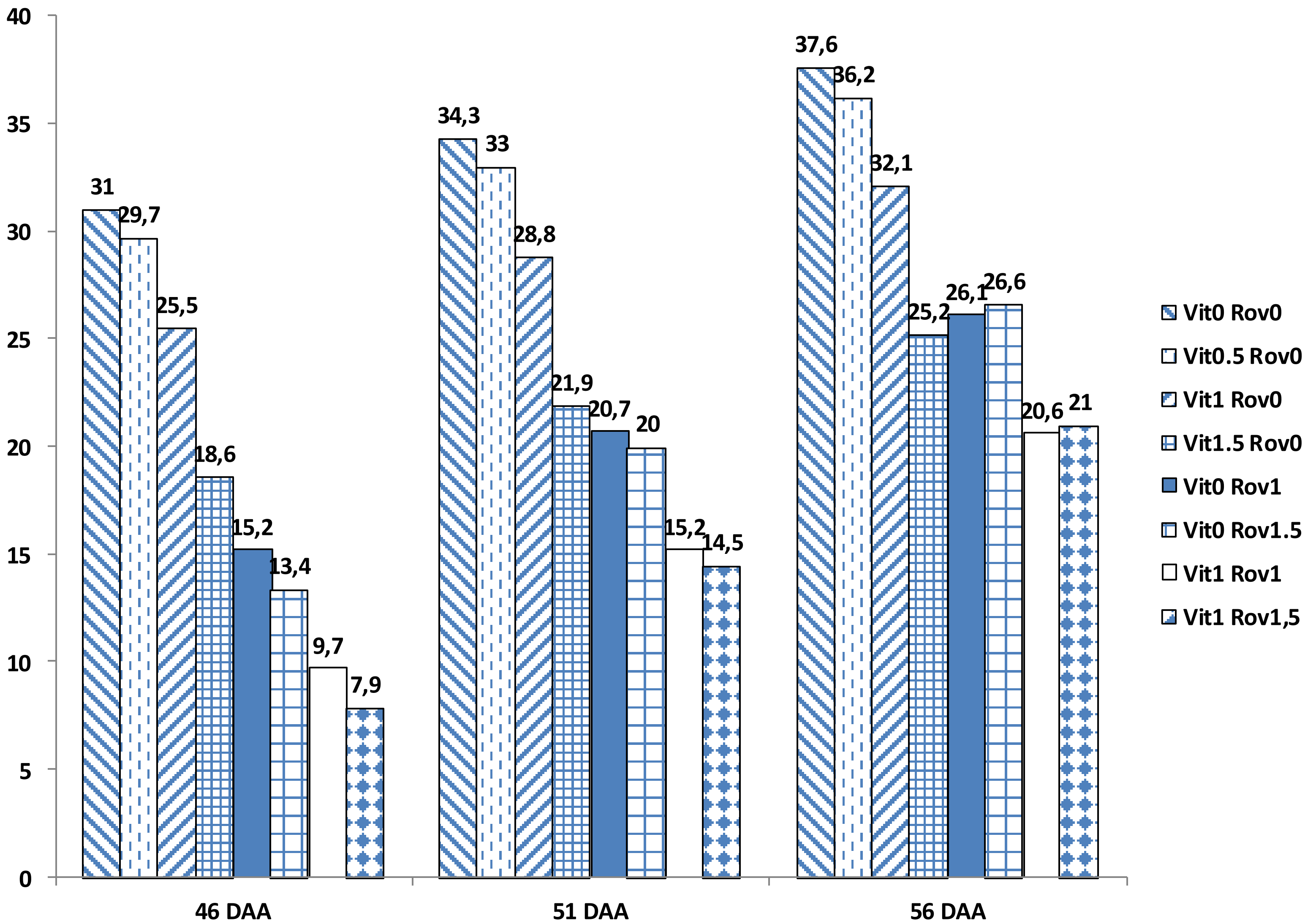


Fig. 6. Differences with respective controls at 46 and 56 days after application in estimated and actual *Botrytis* Incidence with Vitazyme 1.5 l ha^{-1} and Rovral 1.5 kg ha^{-1} , both alone.

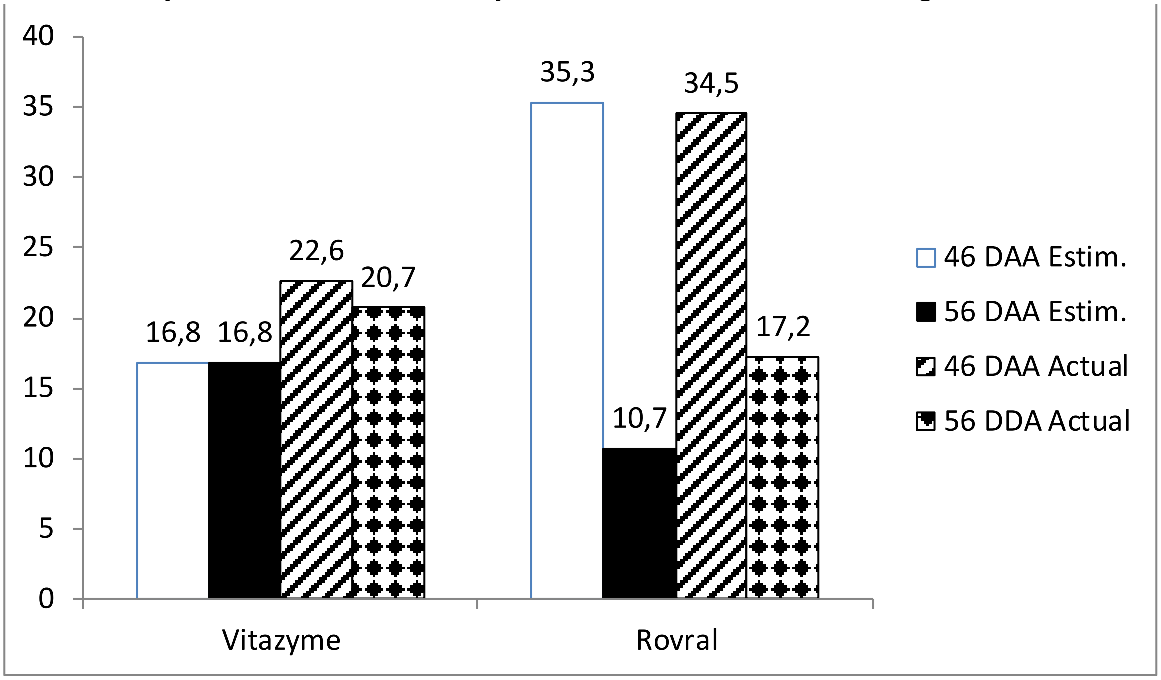
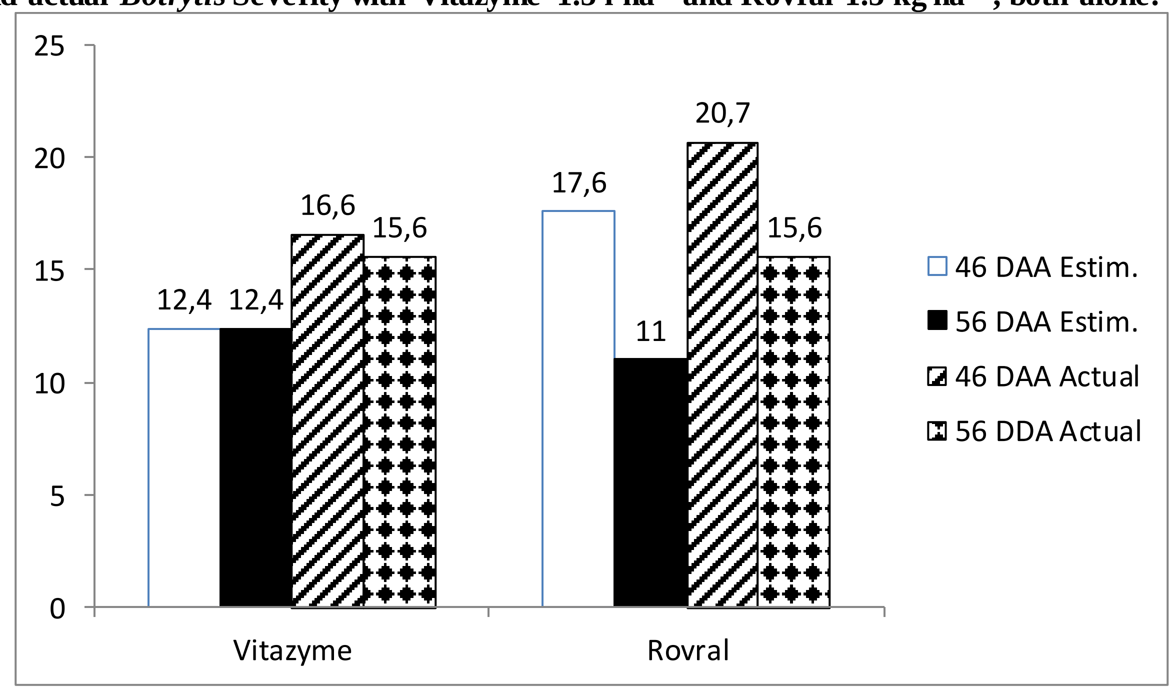


Fig. 7. Differences with respective controls at 46 and 56 days after application in estimated and actual *Botrytis* Severity with Vitazyme 1.5 l ha^{-1} and Rovral 1.5 kg ha^{-1} , both alone.



Quality Parameters: Fruit Brix, Firmness and Yield for Packaging

Vitazyme alone, at the manufacturer-recommended rates of 1 and 1.5 l ha^{-1} (Treatments 2 and 8, respectively) increased significantly, both Brix or % soluble solids (mean increases above the control or dosage zero by 1.40 and 1.92 percentage points), and fruit firmness or consistency by 1.58 and 1.74 Newtons), respectively, which represents marked improvement in fruit quality, while Rovral had no effect whatsoever on these two parameters, in any rate (Table 2, Figs. 8-9).

Note the dented shape of alternate peaks and valleys of Figs. 8 and 9, which, as you may see in Table 2 below, is due to the fact that treatments with pair or even numbers (2, 4, 6 and 8), which are the ones with Vitazyme at rates of 1 l ha^{-1} and 1.5 l ha^{-1}, show higher columns and higher statistical significance in both parameters, and vice versa, treatments with odd numbers (1, 3, 5 and 7), which DO NOT have Vitazyme at either 1 l ha^{-1} or 1.5 l ha^{-1}, show the smaller columns and were statistically smaller.

See also ahead, in Yield and Economic Evaluation, Fig. 11, the sharply higher "yields for packaging" (greatest contrast between pair and odd columns), due to better fruit appearance and consequently higher price, in all the treatments with Vitazyme 1 ha^{-1} and 1.5 l ha^{-1}.

Among the 3 application to evaluation intervals, there were no differences in Brix, while in Firmness the intermediate interval (51 DAA) was significantly higher than 46 and 56 DAA (Table 2).

Table 2. Mean fruit Brix and Firmness by intervals or days after application and treatments of Vitazyme x Rovral.

	Brix (%)	sig.	Firmness (N)	sig.
Application-evaluation (DAA) intervals				
46 days	9.16	a	4.86	b
51 days	9.45	a	7.99	a
56 days	9.48	a	5.32	b
Standard error	0.116		0.156	
Vitazyme x Rovral Treatments				
8 (V1.5, R0)	10.43	a	6.98	a
2 (V1, R0)	9.91	a	6.81	a
4 (V1, R1.5)	10.17	a	6.79	a
6 (V1, R1)	10.00	a	6.67	a
5 (V0, R1)	8.68	b	5.54	b
7 (V0.5, R0)	8.67	b	5.49	b
1 (V0, R0)	8.51	b	5.23	b
3 (V0, R1.5)	8.50	b	4.93	b
Standard error	0.190		0.255	

* Means accompanied by a common letter do not differ significantly by Tukey ($\alpha = 0.05$).

Fig. 8. Brix (%) by treatments Vitazyme x Rovral (according to Table 2)

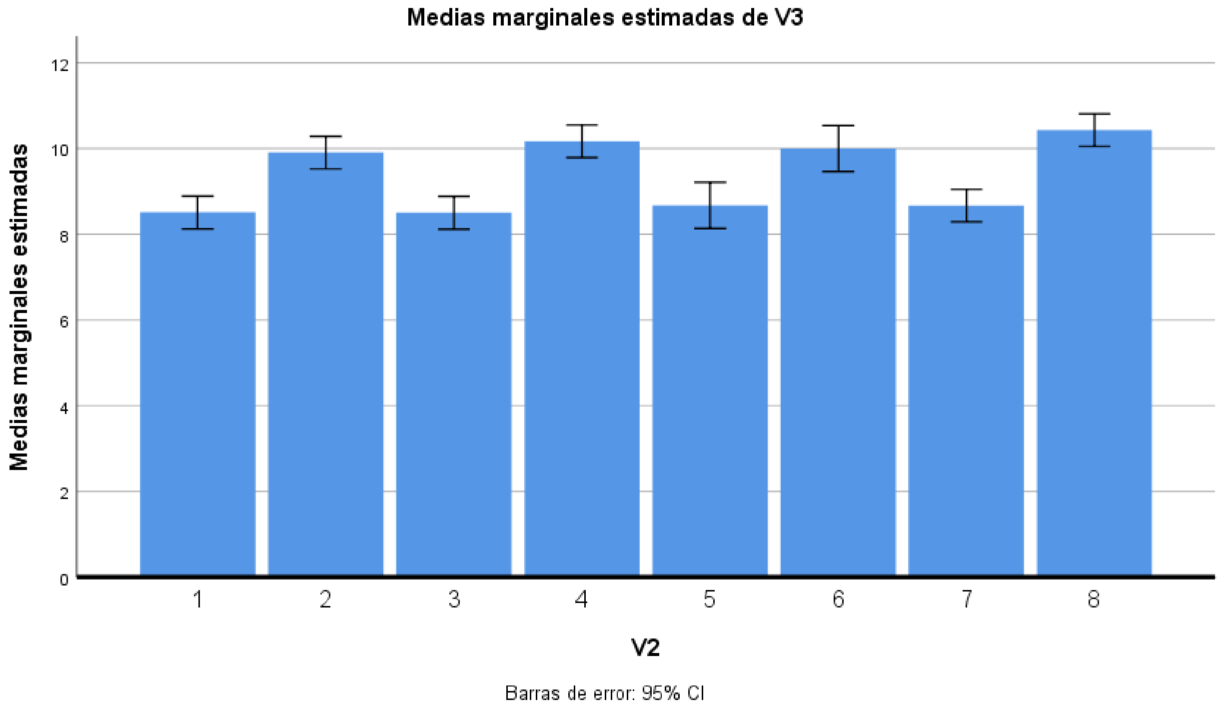
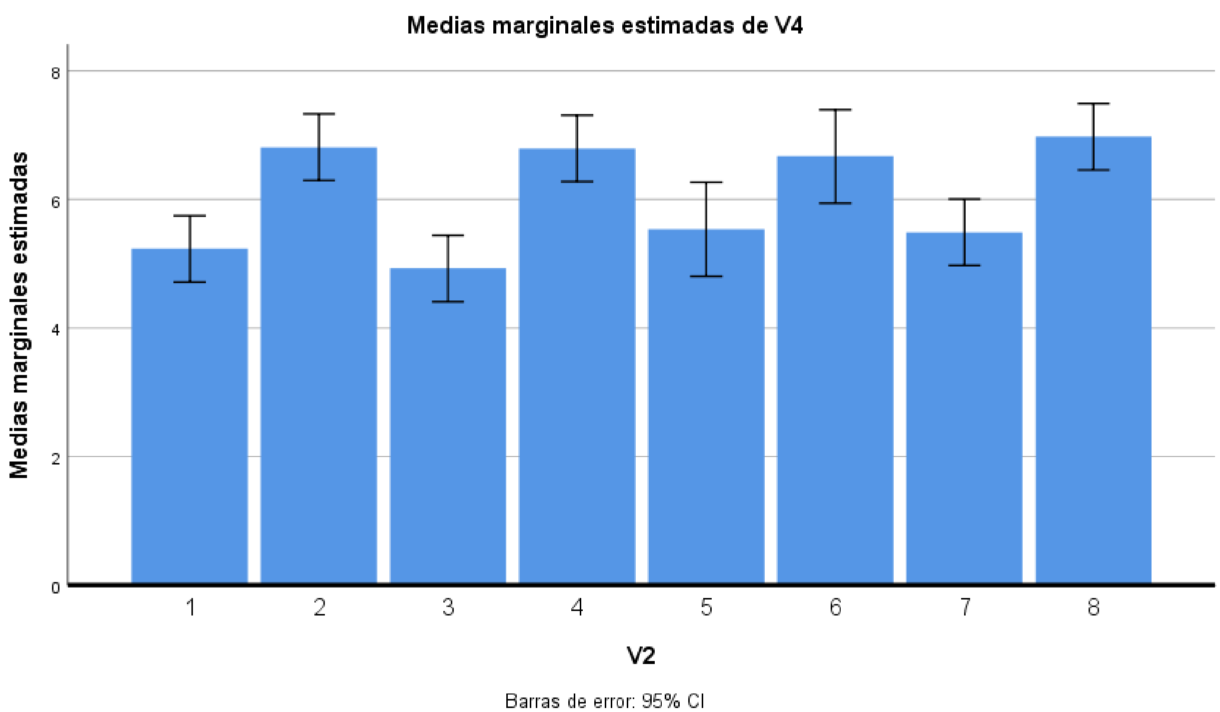


Fig. 9. Firmness (Newtons) by treatments Vitazyme x Rovral (according to Table 2)



Yields and Economic Evaluation

Figs. 10-12 show mean yields of 3 pickings, while Table 3 and Fig. 13 show the economic assessment, including the overall yields of 3 pickings and the income (Mexican pesos per hectare $ MXN/ha), disaggregated in “for process”, “for packaging” and “Total”, by 8 Vitazyme x Rovral treatments; the added value or income, by difference with the absolute control (without Vitazyme and Rovral); the total application cost of the two quoted products, according to rates per treatment; and (also Fig. 13) the net profits per hectare (difference between added value or income minus the total added cost) sorted in descending order according to "Order of Merit".

Note the dented shape of alternate peaks and valleys of Figs. 10-12, which is due to the fact that treatments with even or pair numbers (2, 4, 6 and 8), with Vitazyme rates of 1 l ha^{-1} and 1.5 l ha^{-1}, show the highest columns, and at bottom of Table 3, the higher cumulative returns or income and profits; and vice versa, treatments with odd numbers (1, 3, 5 and 7), which do not have Vitazyme at 1 l ha^{-1} or 1.5 l ha^{-1}, show the lowest columns, and, in upper Table 3, show lowest cumulative yields, income and profits.

Also note is Fig. 11 (Yield for Packaging) a more stark or pronounced contrast between the pair-numbered columns (Vitazyme 1 and 1.5 l ha^{-1}) and the odd-numbered columns (without Vitazyme 1 or 1.5 l ha^{-1}), demonstrating in the former higher quality in fruit appearance and consequent price ($22.50/kg, compared to $10/kg for the Yield for Process).

It also stands out in Table 3 and Fig. 13, that two first treatments in descending value (No. 6 and 4) included both products: Vitazyme at 1 l ha^{-1} and Rovral at 1 or 1.5 kg ha^{-1}, confirming what was observed in *Botrytis* Incidence and Severity: that the best treatments were those in which both products were applied combined, at recommended rates, demonstrating synergistic or boosting effects of Vitazyme on Rovral. It should be added that, as in the disease parameters, there was no improvement in the higher rate of Rovral , but rather the combination of Rovral 1 kg ha^{-1} and Vitazyme 1 l ha^{-1} (the same rate of both) was the best overall treatment (No. 6) in net profits (and therefore, the treatment recommended in the present study) due to its lower cost (because of the lower rate of Rovral) compared to the combination of 1.5 kg ha^{-1} of Rovral with the same rate of Vitazyme 1 l ha^{-1}, which was the second best treatment (No. 4). We also reiterate that the only rate of Vitazyme studied in combination with Rovral was 1 l ha^{-1}, and therefore we cannot predict or recommend combinations with other dosages of Vitazyme.

Finally, stands out, that where Vitazyme was applied alone, at the manufacturer-recommended rates of 1 and 1.5 l ha^{-1}, much higher yields, revenues and net profits were achieved than where Rovral was applied alone. Thus, the rates of Vitazyme 1 l ha^{-1} and 1.5 l ha^{-1} applied alone, produced total yields of 4425 and 4465 kg ha^{-1} and net profits of 11417 and 9555 pesos MXN/ha, respectively, which reached third and fourth places in descending order, immediately after the two treatments in which both products were combined, while that same rates of Rovral alone (1 & 1.5 kg ha^{-1}), produced total yields of 3994 and 3977 kg ha^{-1}, and net profits of 3783 and 1030 MXN pesos/ha, respectively, i.e., net profits were 4.4 times higher (10486 / 2407) on average with Vitazyme alone than with Rovral alone, at the same recommended rates.

Fig. 10. Yield for Process by treatments of Vitazyme x Rovral (according to Table 3)

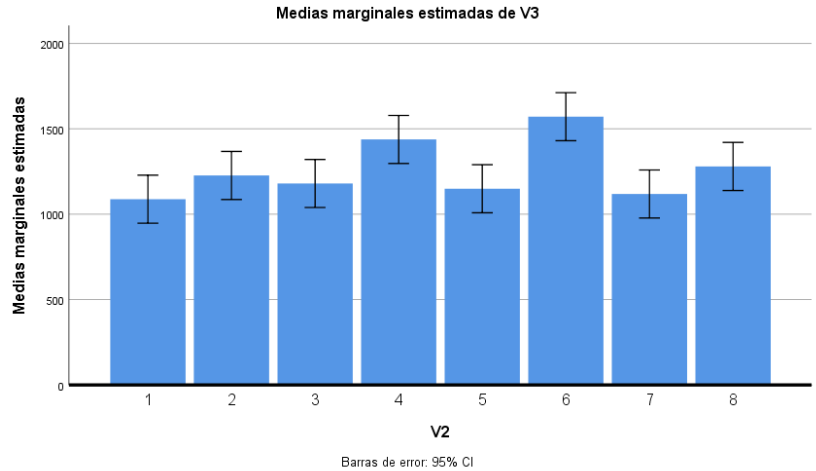


Fig. 11. Yield for Packaging by treatments of Vitazyme x Rovral (according to Table 3)

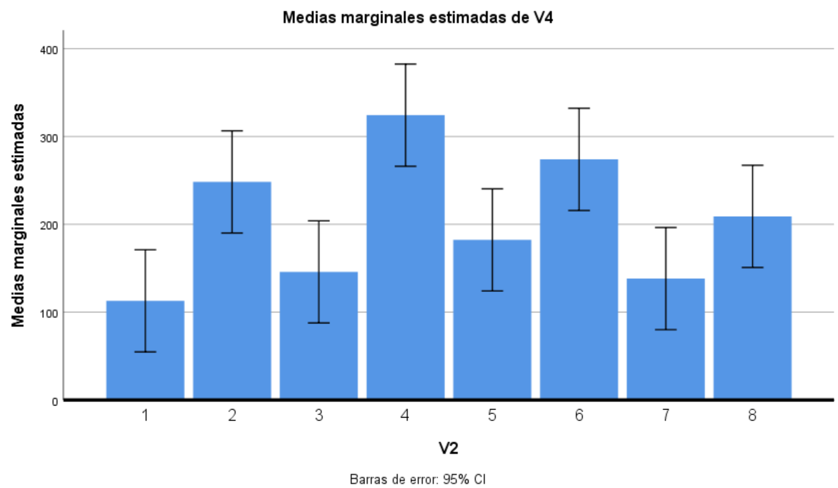


Fig. 12. Total yield by treatments of Vitazyme x Rovral (according to Table 3)

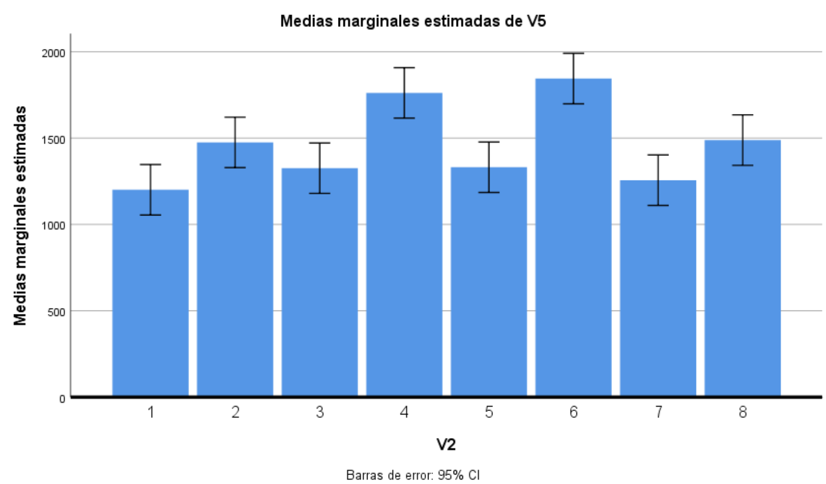


Table 3. Economic Evaluation of Vitazyme x Rovral Treatments.

Treatment	Yield ($kg\ ha^{-1}$)			Paid Income ($ MXN/ha)			Added Income $MXN/ha	Total Cost $MXN/ha	Net profits $MXN/ha	Order Merit
	For process	For packaging	Total	Process at $10/kg	Packaging at $22.5/kg	Total $ MXN/ha				
1 (V0, R0)	3264	339	3602	32635	7617	40253				8
3 (V0, R1.5)	3540	438	3977	35396	9844	45240	4987	3957	1030	7
7 (V0.5, R0)	3354	415	3769	33542	9328	42870	2617	1095	1522	6
5 (V0, R1)	3447	547	3994	34469	12305	46773	6521	2738	3783	5
8 (V1.5, R0)	3838	627	4465	38383	14109	52493	12240	2685	9555	4
2 (V1, R0)	3680	745	4425	36802	16758	53560	13307	1890	11417	3
4 (V1, R1.5)	4313	973	5286	43127	21891	65018	24765	5547	19218	2
6 (V1, R1)	4713	822	5534	47125	18492	65617	25365	4328	21037	1

Public or retail price of Vitazyme: $530 MXN/L, Rovral retail price: $812.60 MXN /L. Labor cost: $100 MXN /ha.

This better yield and profitability of Vitazyme alone than Rovral fungicide alone, could be due that Vitazyme acted in two ways: directly on the strawberry plants as a biostimulant, and indirectly through its effect on the disease, described before, while Rovral only acted in the latter way: on the disease.

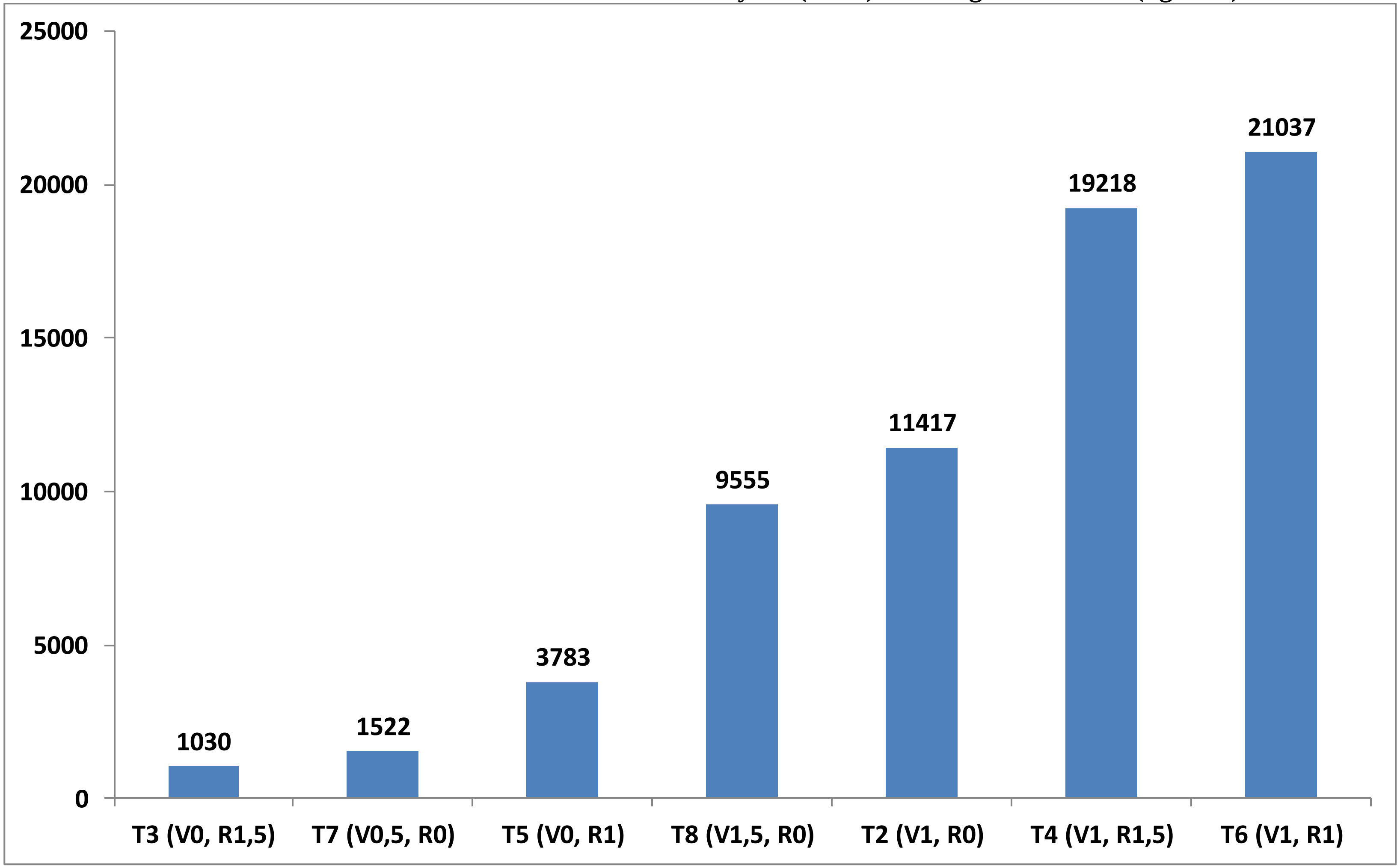
Between application to harvest intervals (DAA) it was noted that yields, both for Process as Total (because “for Process” was the largest component of the “Total”) was highest in the first interval (46 DAA) and lowest in the last (56 DAA), while yield “for Packaging) was reverse: lowest in the first interval and highest in the last (Table 4), i.e.: more quality at later dates (& age).

Table 4. Mean yields per picking for process, for packaging and total by intervals application-evaluation.

Intervals application-evaluation	Yield for Process	sig.*	Yield for Packaging	sig.*	Total yield	sig.*
46 DAA	1464.1	a	151.6	b	1615.6	a
51 DAA	1230.2	b	224.0	a	1454.2	b
56 DAA	1074.2	c	237.5	a	1311.7	b
Standard error	40.18		16.61		41.73	

* Means accompanied by a common letter do not differ significantly by Tukey ($\alpha = 0.05$).

Fig. 13. Net profits (difference with untreated Absolute Control T1, in MXN pesos/ha) of treatments of different rates of biostimulant Vitazyme (l ha^{-1}) and fungicide Rovral (kg ha^{-1}).



CONCLUSIONS

It was demonstrated that the application of natural biostimulant containing four brassinosteroids + triacontanol + three B vitamins (Vitazyme), at the recommended rate of 1 l ha^{-1}**,** combined with iprodione fungicide (Rovral PH 50), at the recommended rate of 1 kg ha^{-1}, in three fortnightly (15 day) interval, foliar applications, showed lowest percentages of incidence and severity of Grey Mold (*Botrytis cinerea* Pers.) and highest yields, incomes and net profits in strawberry crop, since they have additive or synergistic effects: Vitazyme enhances the effect of fungicide Rovral, as well as bringing about yield and quality biostimulant effect.

On the other hand, Vitazyme, at rates of 1 l ha^{-1} and 1.5 l ha^{-1} shows very marked and significant increases in the percentage of brix or soluble solids, and, therefore, fruit juice sweetness, and higher fruit firmness or consistency, in Newton, as well as a much higher proportion of fruits picked for packaging (of higher price), while Rovral fungicide, had no effect in any rate, on any quality parameter.

Resistance inducers, as in the present thesis Vitazyme, are not necessarily a replacement for the traditional fungicides. Their alternate use or in combination with these pesticides can reduce the number of applications or perhaps the application rate. This could reduce total costs and the pollutant load, in addition to helping extend the durability of resistance in cultivars with genes for resistance to strains of pathogens.

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