Influence of "effective microorganisms" (EM) on vegetable production and carbon mineralization - A preliminary investigation

M.J. Daly

D.P.C. Stewart

ABSTRACT. The influence of effective microorganisms (EM), a commercially available microbial inoculant containing yeast's, fungi, bacteria and actinomycetes, was evaluated in field trials of commercially produced, irrigated vegetable crops on "organic" farms in Canterbury, New Zealand during 1994-1995, and in a laboratory incubation. EM plus molasses were both applied, at 10 L ha^{-1} in 10 000 L ha^{-1} water, three times to the onions, twice to the peas and seven times to the sweetcorn. EM plus molasses increased the onion yield by 29% and the proportion of highest grade onions by 76%. EM plus molasses also increased pea yields by 31% and sweetcorn cob weights by 23%. A four week incubation at 30°C of loamy sand and 1% w/w pasture litter had treatments including a control, glucose, and EM plus glucose, and captured respired carbon (C) using NaOH traps. By the end of the incubation the glucose treatment had respired 38% more C than the control. The EM treatment respired an additional 8% more C than the glucose treatment. Using EM stimulated C mineralization in the laboratory incubation, but a corresponding increase in mineralization of organic nitrogen, phosphorus and sulphur was not measured.

Keywords: effective microorganisms; vegetable yield; peas; sweetcorn; onion; carbon mineralization

Mike Daly and Dean Stewart are with the New Zealand Pastoral and Agricultural Research Institute(AgResearch), PO Box 60, Lincoln, Canterbury New Zealand.

Address correspondence to : Mike Daly, Email: dalym@agresearch.cri.nz

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INTRODUCTION

A healthy resilient soil is imperative for sustainable agriculture and is achieved in part through crop rotations and the recycling of organic wastes maintaining adequate soil organic carbon (Parr *et al*., 1992). Soil microorganisms mineralize organic matter releasing plant available nutrients and promoting the stabilization of the recalcitrant residues into humus. Kyusei Nature Farming, a Japanese system of "organic" farming, promotes sustainable agriculture by encouraging the use of crop residue recycling, green manures and balanced crop rotations so external inputs such as chemical fertilisers and pesticides are unnecessary (Higa, 1996). They also promote the use of Effective Microorganisms (EM; International Nature Farming Research Centre, PO Box 26, Atami 413-91, Japan), a liquid microbial inoculant containing fungi including yeast's and bacteria including actinomycetes, lactic acid and photosynthetic bacteria (Table 1; Higa, 1994; 1996). Reportedly, following EM application, there is an increase in soil microorganisms that are beneficial for plant growth that results in: more rapid organic matter mineralization; suppression of soil borne disease pathogens; and increased crop yield and quality (Asia-Pacific Natural Agriculture Network, 1995). EM is generally applied with a carbon and energy source (i.e. molasses) for microorganisms.

Some EM research and field testing has been done in the Asia-Pacific region (e.g. Myint, 1994; Sangakkara, 1994; Sangakkara & Higa, 1992). EM was used for the first time on crops in New Zealand during the 1994-95 growing season. A research program, established in 1994, used on-farm trials and field days, and aimed to increase the number of "organic" growers and volumes of "organic" produce from New Zealand. The

approach was to involve farmers in the trials and to keep the research simple using readily available technology. Although these field trials evaluated several fertilisers, only the EM plus molasses and control treatments are presented here.

Reportedly one mechanism by which EM increases plant growth is by increasing the rate of organic matter mineralization which results in an increase in plant-available nutrients (Asia-Pacific Natural Agriculture Network, 1995). Piyadasa *et al*. (1995) investigated nitrogen (N) and phosphorus (P) release from soil amended with organic matter over a 21 day incubation at 60°C. There were increased concentrations of inorganic N and P as a result of applying EM solution compared with a control.

The objectives of this research were to evaluate the influence of EM on vegetable yields, and to determine if, under controlled conditions, EM would stimulate C mineralization.

MATERIALS AND METHODS

Field trials

These were sited within 25 km of Lincoln University, Canterbury, New Zealand (latitude 43^{o} 39' south, longitude 172^{o} 27' east, altitude 50 m asl), on commercial farms and run in partnership with the farmer who cultivated, planted and managed the crop, and assisted with taking measurements. Soil chemical analysis included "Quick-test" soil fertility analysis, a standard New Zealand method of soil analysis (Lee *et al*., 1991). The crops all received irrigation when the farmer determined it was necessary.

The onion crop (*Alium cepa* cv. "Pukekohe Long Keeper") was grown on a "Wakanui" silt loam soil (Kear *et al*., 1967) which was prepared by rotary hoeing after a winter green feed crop in August. The field had previously been in ryegrass/white clover pasture for four years. Soil test values on 5 September for 0-15 cm depth were: pH 5.8; and quick test values of, phosphorus 15; sulphate-sulphur 9; potassium 5; magnesium 19 and calcium 8 (Lee *et al*., 1991). The trial was direct seeded using a precision seeder in 1.5 m wide beds with four rows to the bed using a 300 mm between row spacing. Seed spacing within the row was 60 mm. The trial had four replicates and a plot size of 5 by 1.5 m. EM (Table 1) was applied at a rate of 10 L ha^{-1} with 10 L ha^{-1} of molasses mixed into water and applied, approximately 24 hours after mixing, at 10 000 L ha^{-1} through a watering can onto the foliage of the crop, on November 11, December 22 and January 20. This equates to 1 mm of water and 4.3 kg C ha^{-1} in the molasses for each application. Crop vigour was accessed visually at bulbing (January 22). At harvest (March 7) the field dried onions were graded and weighed.

The process pea crop (*Pisum sativum* cv. "Princess") was grown on a Templeton silt loam soil (Kear *et al*., 1967) which was ploughed after an oat grain crop. The straw was incorporated by cultivation during the winter. Soil tests on 6 October for 0-15 cm depth were: pH 6.3; and quick test values, phosphorus 15; sulphate-sulphur 13; potassium 8; magnesium 21; and calcium 14 (Lee *et al*., 1991). The peas were sown on 7 October at 290 kg ha^{-1} using a 15 cm between row spacing. In addition the field had a basal application of 250 kg ha^{-1} of reactive phosphate rock applied to correct a low soil phosphate concentration. The trial had four replicates and a plot size of 5 by 10 m.

EM was applied at the previously described rate twice during crop growth (at mid flower and at early pod development). The crop was irrigated on the 16 and 23 December, and harvested on the 31 December. A composite tissue sample was taken from each of the treatments at full flower for chemical analysis.

The sweetcorn (*Zea mays* var. *convor saccharata* cv. "Honey and Pearl") was grown on a Wakanui silt loam soil (Kear *et al*., 1967) which had been intensively managed using raised beds with a six year rotation of vegetable crops. The previous crops were peas and beans. Soil tests in September for 0-15 cm depth were: pH 6.8; and quick test values, phosphorus 191; sulphate-sulphur 4; potassium 41; magnesium 60 and calcium 18 (Lee *et al*., 1991). An unreplicated split-plot design was used with three sowing dates (October 18, November 24, December 19) as main-plots, and two foliar applied treatments (EM and a control i.e. water only) as sub-plots. Sub-plot size was 4 by 1 m (3 rows of corn). The EM was applied as previously described. The control treatment received water at 10 000 L ha^{-1}. Applications were made after plant emergence at 10-15 day intervals throughout the growing season up until flowering, for each sowing date. This gave an average of seven foliar applications per sowing date. The sweetcorn plants from each sowing date were harvested when they reached fresh maturity. Ten plants per plot were randomly selected and components (whole plants, number of stems, number of cobs) measured.

Laboratory incubation

The soil used for the incubation was from the A horizon of a Selwyn loamy sand (a moderately fertile recent soil, Kear *et al*., 1967). The soil sample (0-5 cm) was taken after the vegetation and the surface litter was removed and was sieved through a 2 mm sieve. The soil water content was adjusted to 26% and was preincubated for four days at 30^{o}C and sieved again (4 mm) prior to the incubation. The equivalent of 50 g oven dry soil, was weighed into gas-tight glass jars for the incubation and 1% w/w ground pasture litter was added to all jars. The pasture litter, mainly white clover (*Trifolium repens*) and ryegrass (*Lolium perenne*), was dried at 80^{o}C and ground (500 μm).

The treatments were a control, a glucose and an EM plus glucose treatment. All treatments received 1 mL of liquid prior to the incubation. The control received water, the glucose treatments received 1 g L $^{-1}$ glucose solution, and the EM treatment received 0.1% v/v EM and 1 g L $^{-1}$ glucose solutions. This is equivalent to an application rate of 10 000 L ha $^{-1}$ (based on the hypothetical surface area of a 5 cm deep column of soil of 50 g mass at a bulk density of 1.1 g cm^{-3} i.e. the bulk density of the field soil), the same rate applied to the field crops.

Glass jars containing CO_2 traps, plastic containers holding 20 mL of 1M NaOH mounted on a wire stand, were sealed and incubated at 30^{o}C. There were four replicates of each treatment. Each week the jars were taken out of the incubator, and the traps rapidly removed and sealed (to avoid CO_2 contamination). The jars were left with the lids off for approximately an hour to avoid anaerobia. Traps with fresh NaOH solution were placed into the jars and the jars resealed. Analysis of the traps was carried out

using a method adopted from Anderson (1984) titrating with 1M HCl after the addition of $BaCl_2$ to the NaOH (to precipitate the carbonates) using phenolphthalein indicator. Soil analysis was done on air dried, sieved soil (2 mm) and included pH using a 1:2.5 soil to water ratio, electrical conductivity using a 1:5 soil to water ratio, and mineral-N (ammonium and nitrate), Olsen-P and sulphate-S as described by Lee *et al*. (1991).

RESULTS

Crops

EM plus molasses caused a significant yield increase over the untreated control and produced more first grade onions (Table 2). The yield of peas was increased by EM plus molasses (Table 3). There were no major differences in the herbage nutrient concentrations of peas between treatments (Table 3) and no nutrients were deficient (Clarke *et al*., 1986). The weight of sweetcorn cobs was significantly increased by EM plus molasses ($P<0.05$, Table 4). There was variability in the sweetcorn plant weight data, however, a consistent trend of increased plant weight following applications of EM plus molasses was evident across all sowing dates (Table 4). The numbers of tillers and cobs were not significantly influenced by EM plus molasses applications (Table 4).

Laboratory incubation

During the first week of the incubation C was rapidly respired at a similar rate for all treatments, after which the rate of C mineralization decreased (Fig. 1). The cumulative amount of C mineralized was significantly greater than the control by 38 and 49% respectively for glucose and EM plus glucose treatments (Fig. 1). There was a

significant increase in the cumulative amount of C mineralized by approximately 8% resulting from applying EM (i.e. over the glucose treatment). This 8% net increase from applying EM was substantially greater than the amount of C added in EM (i.e. 6.7 and 0.01 mg C respectively).

The treatments had little influence on the soil pH and mineral-N, Olsen-P and sulphate-S concentrations but both glucose and EM plus glucose treatments had a greater soil EC (Table 5), which would be unlikely to influence plant growth.

DISCUSSION

This work was a preliminary investigation. The field experiments were designed to evaluate a range of fertilisers for crop production, not to focus solely on the effects of EM. Additional treatments of water (for the onion and pea crops) and molasses would have been useful as additional controls, to measure the effects of water and carbon on the crops. However, the amount of water applied with the EM to the onion and pea crops was small (i.e. 3 and 2 mm respectively) and was therefore unlikely to influence an irrigated crop. Other work has reported that applying C to crops causes N immobilization and reduces plant growth (e.g. Bååth *et al*., 1978; Ritz and Griffiths, 1987), so if anything the small amount of molasses applied would increase indigenous microbial activity and reduce crop yield.

Applications of EM plus molasses did have a beneficial effect on the three crops grown, demonstrating the potential of EM for improving the yield and quality of a range of

vegetable crops. Higa (1994), Myint (1994) and Sangakkara (1994) have also reported that EM improved crop quality and yield.

It is not possible from the field experiments reported in this paper to determine the mechanism(s) by which EM plus molasses influenced plant growth. However, the application of EM to soil/litter mixtures stimulated C mineralization in the laboratory (Fig. 1). However, there was no direct evidence of increased nitrogen, phosphorus or sulphur mineralization in the soil at the end of the incubation, or increased nutrient uptake from the pea crop data. Mineralization of C by soil microoganisms generally results in increased concentrations of nitrate, sulphate and phosphate in soils in the long-term. But in this laboratory incubation it appears that the microbial population was growing and hence was immobilizing (i.e. removing inorganic nutrients from the soil to use for their growth) these nutrients over the four weeks of the incubation.

While stimulating soil organic matter (SOM) mineralization is desirable in the short-term to provide nutrients for plants, continued rapid SOM mineralization is not desirable in the longer-term as a depletion of SOM would be unsustainable. SOM enhances soil quality by promoting soil structure and cation retention. If repeated use of EM caused a substantial reduction in SOM it would undesirable. However, it is most likely that EM increases mineralization of relatively labile SOM and is therefore likely to have less effect if it is used repeatedly and the amount of labile SOM is reduced. However, long-term repeated use of EM may need to be accompanied by additions of OM such as compost. Compost, because it has been biochemically stabilized, is useful for increasing

SOM and providing plant nutrients (e.g. Stewart *et al*., 1998abc). It is recommended practice to apply OM with EM (Asia-Pacific Natural Agriculture, 1995) and hence this technology may suit "organic" growers because they commonly use green manures and composts as part of there crop management program. The use of EM accompanied by additions of OM may provide long-term sustainable systems with improved nutrient mineralization and plant uptake.

More research needs to be done to determine the long-term influence of EM on organic matter mineralization by either using longer laboratory incubations or field trials in conjunction with a test crop to determine plant nutrient uptake under field conditions. Research on the repeated use of EM could also be done. Research needs to determine if EM stimulates plant growth by other mechanisms.

CONCLUSIONS

EM plus molasses increased onion and pea yields and sweetcorn cob weights in the field. EM plus glucose increased C mineralization in a laboratory incubation. More research is required to determine the mechanisms by which EM influences plant growth including the mineralization of organic plant nutrients.

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TABLE 1. List of species and concentrations in EM concentrate.

Species[1]	Minimum viable organisms per mL
Streptomyces albus	10^5
Propionibacterium freudenreichil	10^5
Streptococcus lactis	10^5
Aspergillus oryzae	10^5
Mucor hiemalis	10^5
Saccharomyces cerevisiae	10^5
Candida utilis	10^5

[1] EM also includes an unspecified number of *Lactobacillus sp, Rhodopseudomonas sp, and Streptomyces griseus*

TABLE 2. Influence of EM plus molasses on onion mid season vigour, yield and grade

Treatment	Vigour Score[1] (1-5)	Total Yield (t ha^{-1})	First grade (t ha^{-1})	Process grade (t ha^{-1})	Small grade (t ha^{-1})
Control`	2.6	42	25	11	6
EM + molasses	3.0	54	44	6	4
$LSD_{P=0.05}$	*0.8*	*11*	*13*	*6*	*3*

[1] A high score indicates high vigour

Table 3. Influence of EM plus molasses on pea herbage chemical analysis[1] and yield

Treatment	N	P	K	S	Mg	Ca	Yield (t ha^{-1})
	%	%	%	%	ppm	ppm	at 105 TR[2]
Control	3.46	0.29	1.34	0.26	0.20	0.76	6.1
EM + molasses	3.46	0.32	1.46	0.24	0.21	0.74	8.0
$LSD_{P=0.05}$	-	-	-	-	-	-	*1.4*

[1]Unreplicated composite samples, [2]TR = Tendorometer reading

TABLE 4. Influence of EM plus molasses and sowing date on sweetcorn growth

Sowing Date	Treatment	Whole Plant Weight[1] (kg)	Tiller No.	Cob No.	Cob Weight[1] (g)
October	EM + molasses	1.06	2.6	1.5	440
	Control	1.04	2.2	1.7	370
November	EM + molasses	1.02	2.6	2.0	380
	Control	0.89	2.7	1.8	310
December	EM + molasses	1.12	2.4	1.9	400
	Control	0.93	1.7	1.2	300
Overall Mean	EM + molasses	1.07	2.5	1.8	400
	Control	0.95	2.2	1.6	330
$LSD_{P=0.05}$	-	*0.21*	*1.0*	*1.1*	*45*

[1] Fresh Weight

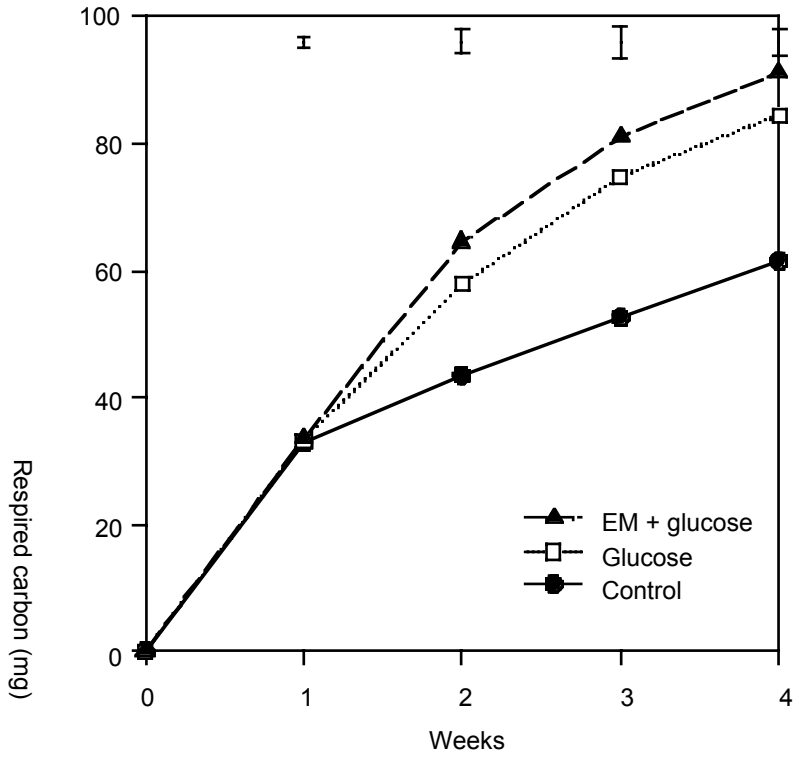
TABLE 5. Influence of glucose and EM plus glucose on soil pH, electrical conductivity (EC), mineral-N, Olsen-P and sulphate-S at the end of the incubation.

	Control	Glucose	EM + glucose	LSD (*P*=0.05)
pH	6.2	6.1	6.2	0.2
EC (mS cm^{-1})	0.17	0.19	0.19	0.01
Mineral-N[1] (μg g^{-1})	112	108	110	14
Olsen-P (μg mL^{-1} soil)[2]	13.0	13.0	13.0	1.2
Sulphate-S (μg g^{-1})	13.0	13.5	13.8	1.9

[1] Nitrate plus ammonium, [2] Lee *et al*. (1991).

FIGURE CAPTION

FIGURE 1. Influence of EM on the cumulative amount of carbon respired (bars are LSDs *P*=0.05).


100
80
60
40
20
0
Respired carbon (mg)
0
1
2
3
4
Weeks
EM + glucose
Glucose
Control