FINAL REPORT
New York State Onion Research and Development Program
(Marketing Order)
2012-2013

Role of Adjuvants in Bacterial Diseases of Onions

Funding Period and Amount Allocated:
April 1, 2012 to March 31, 2013 - $3,000

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ACKNOWLEDGMENTS:
Field support staff: Katie Klotzbach and Elizabeth Buck, Cornell Cooperative Extension Vegetable Program, Albion and Batavia, respectively
Technicial support and product supply: Chris Daum, Helena Chemical, Albion, and Joe Palmer, Crop Protection Services, Fancher

BACKGROUND:

Bacterial diseases of onions have become an increasing threat to the sustainability of the New York onion industry with losses of 40% or more occurring in some lots in some years. If bacterial diseases of onion could be managed effectively, this could save New York onion growers millions of dollars annually. Management of bacterial diseases of onions is a high research priority for the Onion Research and Development Program (ORDP) as there are few effective management strategies.

In New York, onions are plagued by several bacterial pathogens that cause both bulb and leaf decay. The most common pathogens identified to cause bacterial bulb decay in onion include *Burkholderia cepacia*, *Pantoea ananatis*, *P. agglomerans, Enterobacter cloacae* and most recently, *Rahnella* species. These pathogens have also been found to commonly occur in NY soils where onions are grown. Some are known to enter the plant via the leaves and neck, and from there, infection progresses down into the bulb. Because bacterial bulb decay often affects only a single internal scale while the outer scales remain firm, such infected bulbs are virtually impossible to detect. Such onions are shipped and consequently rejected, which often results in entire loads being dumped, despite only a small percentage of bulbs being infected.

Ultimately, an integrated pest management approach will be required to manage bacterial diseases of onions, which could involve field sanitation, tolerant varieties, induced resistance materials and bactericides, crop rotation and cover crops, reduced nitrogen fertility and post-harvest techniques including strategic curing conditions and imaging technology. Researchers and Extension professionals at Cornell and Nationwide have been working on several of these components. Recently, a New York onion grower suggested that the penetrating surfactants that Cornell entomologists strongly recommend to improve efficacy of insecticides for onion thrips control might be allowing for easy entry of bacterial pathogens into the leaves, thus increasing incidence of bacterial bulb decay.

An adjuvant is any substance that is added to the spray tank to improve pesticide activity or application characteristics. Surfactants are a type of activator adjuvant that primarily reduces the surface tension between the spray droplets and the leaf surface, allowing the pesticide to come into closer contact with the leaf surface, thus aiding in absorption. Surfactants can be nonionic, anionic, cationic or organosilicones, and can have both spreader and penetrating properties. Vegetable crop oil concentrates are another type of activator adjuvant that promotes the penetration of the pesticide through the waxy cuticle of the plant leaf. Because the insecticides for onion thrips control including Movento, and Agri-Mek and Radiant, have systemic and translaminar activity, respectively, it is very important that they be accompanied by a penetrating surfactant in the spray tank to ensure their best performance. For example, in 2011 Nault showed that addition of surfactant, Induce at 0.5% v/v improved efficacy of Movento by 300%. Since penetrating surfactants can aid in moving pesticide molecules through the waxy cuticle and into the leaf, perhaps bacteria present on the leaf surface of an onion plant might somehow be aided in movement through the waxy cuticle and into the leaf intercellular spaces or into stomatal cavities, facilitating infection that would not have occurred without the surfactant.

A study conducted at Auburn University in Alabama (Zidack et al. 1992) demonstrated that Silwet L-77, a nonionic organosilicone surfactant, could assist entry of the halo blight pathogen, *Pseudomonas syrinae* pv. *phaseolicola* into bean plants. High levels of disease were achieved under hot and dry conditions when the pathogen was applied with Silwett L-77, but, no disease was observed when the pathogen was applied without Silwet L-77. Organosilicone surfactants are known to facilitate stomatal penetration of the leaf surface by aqueous solutions. Delivery of pathogenic bacteria directly into stomata would provide the bacteria with immediate protection from environmental hazards such as ultraviolet and infrared radiation, and desiccation. The substomatal chamber is a natural location for bacterial colonization and infection of the leaf tissue.

In New York, incidence of bacterial bulb decay at harvest was assessed in an onion thrips research field trial conducted by Nault and Hsu in 2010. When the data were pooled across treatments that were sprayed for onion thrips, the sprayed plots had 9.2% bacterial bulb decay, which was twice as much as in the unsprayed plots (4.9%). The plots that were sprayed for thrips received eight applications of Radiant plus Induce, a penetrating nonionic surfactant. Although this trial was not designed to determine the effect of adjuvants on bacterial diseases, these data strongly suggest that the use of adjuvants may play an important role in the development of bacterial diseases of onions. **The role that various adjuvants play in the development of bacterial diseases of onions is a matter that warrants further investigation.**

OBJECTIVE:

To determine whether the use of adjuvants increases the incidence of bacterial diseases of onions.

MATERIALS AND METHODS:

Two on-farm small-plot field trials were set up to evaluate the role of adjuvants on bacterial diseases of onions. One trial was located in Albion, NY in mineral soil in a field cropped to 'Candy' onion transplants, and the other was located in the Elba muck land in a field of 'Red Bull' direct seeded onions. The adjuvants evaluated are described (Table 1):

1) a representative non-ionic surfactant (NIS), **LI700** (Loveland Products, sold by Crop Protection Services, CPS)

2) a representative vegetable crop oil, **Methylated Seed Oil, MSO** (manufactured for Helena)

3) a representative organosilicone, **Kinetic** (manufactured for Helena)

4) commonly used adjuvant, **Dyne-Amic** (manufactured for Helena), a vegetable crop oil and organosilicone blend

5) commonly used adjuvant, **HiWett** (Loveland Products, sold by CPS), an organosilicone blend
Also,
6) **distilled water** containing no chlorine as a control.

Pathogens known to cause bacterial bulb decay in onions including *Burkholderia cepacia*, *Pantoa ananatis* and *Enterobactor cloacae*, were applied in combination with water and adjuvants at a concentration of 1 x 10^8 cfu/ml. The field trials were set up as a randomized complete block design with 6 treatments and 4 replications. Each treatment replicate consisted of enough feet (Albion: 12 feet; Elba: 10 feet) of a single row of onions to treat at least 20 plants. A guard row was left untreated between the treatment plots. Treatments were made using a CO_2 backpack sprayer with a single 8004 VS nozzle, 28 psi and either 80 or 120 gpa (Table 2).

Bacteria were applied via a spray application until runoff from the onion leaves as standard protocol. With water, this was accomplished with 3 passes over the onions at 40 gpa for a total of 120 gpa. Unfortunately, when adjuvant was applied at 0.5% v/v, this resulted in 3X the labeled rates of LI700, MSO and Dyne-Amic, 12X rate of Kinetic and 24X of the rate of HiWett. These "high" rates were applied on Jun-29 and Jul-13 in Albion and on Jul-7 in Elba (Table 2). For the remainder of the applications, rates and volume were adjusted to reflect labeled rates of adjuvants. To acquire the rate of 0.5% v/v for LI700, MSO and Dyne-Amic, 2 applications of 0.25% v/v solution at 40 gpa (X2 = 80 gpa) were made. To acquire the rate of 0.125% v/v for Kinetic, 2 applications of 0.0685% v/v solution at 40 gpa (X2 = 80 gpa) were made. To acquire a rate of 3.2 fl oz per 40 gpa (= 0.0625% v/v) for HiWett, 2 applications of 0.03125% v/v at 40 gpa (X2 = 80 gpa) were made. These "standard" rates were applied on Jul-26 and Aug-9 in Albion, and on Jul-20, Aug-3 and Aug-9 in Elba (Table 1 & 2). Only a single treatment was applied at each location on these dates. Additionally, treatments without bacteria were applied on Aug-9 in Elba, and 2 and 3 applications of adjuvant/water were made without bacteria followed by a final treatment with bacteria in Albion, and Elba, respectively.

Previous studies had shown that leaves of onion plants wounded/stabbed with a florist frog become infected when they are artificially inoculated with bacteria. Thus, we confirmed pathogenicity of our bacterial inoculum, by treating a sample of 16 plants selected at the front of the trial on each spray date by stabbing the inner leaves with a florist frog and then spraying with water plus bacteria.

On Jul-13, 2 weeks after the first "high" rate application in Albion, leaf damage was estimated on a per treatment replicate basis, on a scale of 0 to 100% of the onion leaves being damaged. All of the treated bulbs per treatment replicate were harvested on Aug-29 in Albion and on Aug-21 & 23 in Elba. At harvest, all of the onions in each treatment-replicate were pulled, removed from the field and placed on the ground in a single layer to windrow in a barn without sides. Additionally, onions were pulled from four randomly selected 10-foot sections of row from the field immediately surrounding each trial, to determine "back ground" levels of bacterial bulb decay. On October 15 & 16, onions were topped and the bulbs individually squeezed for softness. Soft onions were cut open to confirm presence of rot. In this process, onions with an internal dry scale extending into the bulb from the neck were often identified. Such bulbs were also recorded, but later determined to not be caused by bacterial pathogens. A sub-sample of 2-3 slightly rotten bulbs per treatment per replicate were collected from the Jul-26 and Aug-3 treatments at Albion and Elba, respectively, for bacterial pathogen identification in the laboratory at Cornell.

Differences among treatments were determined by General Analysis of Variance (ANOVA) and Fisher's Protected LSD test with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Occurrence of bacterial bulb decay (Table 3 &4): The varieties Candy and Red Bull were specifically chosen for this project, because the researchers had gotten substantial levels of bacterial bulb decay in these varieties in previous studies. The back ground levels of bacterial bulb decay at the Albion and Elba trial locations were 6.1% and 11.3%, respectively. In Elba, the trial was located in a field of muck soil that had been mono-cropped to onions for decades. This field was not irrigated. In Albion, the trial was located on mineral soil on ground that had never been cropped to onions and was sprinkler irrigated with a pivot. Onion thrips pressure and their feeding damage to the onion leaves, and Iris yellow spot virus were much higher in Elba than in Albion. The bacterial pathogen, *Burkholderia cepacia*, was detected in soil collected from the Albion site. Although soil samples were not collected specifically from the trial location in Elba, muck soils from the Elba muck land consistently test positive for *B. cepacia*. Higher levels of bacterial bulb decay resulted in the Elba trial (trial average: Jul-20 – 43.3%; Aug-3 – 21.2%; Aug-9 – 18.4%) than in the Albion trial (trial average: Jul-26 – 12.2%; Aug-9 – 7%). The highest rate of wound-induced bacterial bulb decay was initiated on Jul-26 in Albion and on Aug-3 in Elba.

Single application of standard rate of adjuvant with bacteria (Table 3 & 4). No significant differences occurred among treatments except on Aug-9 in Elba (Table 4). Here, MSO had significantly the least bacterial bulb decay, which was not significantly different than LI700. Numerically, Kinetic, Dyne-Amic and HiWett had higher incidence of bacterial bulb decay than the water treatment. ***Our results showed that a single spray application of adjuvant in combination with bacteria did not increase incidence of bacterial bulb decay over water in combination with bacteria.*** No significant differences occurred among treatments for incidence of dry scale, and there were no correlations between dry scale and bacterial bulb decay.

Interestingly, incidence of bacterial bulb decay increased 1.3X to 3.3X from back ground levels when water containing bacteria was sprayed on onions. There was no increase in incidence of bacterial bub decay above the back ground level when water without bacteria was sprayed on onions (Table 5). ***This begs the question whether sources of water used to irrigate and spray onions with is contaminated with pathogenic bacteria, and if so, is it a cause for bacterial bulb decay?***

Single application of high rate of adjuvant with bacteria (Table 5). Two weeks after a single application of adjuvant + bacteria, leaf damage in the form of die back and necrosis was significantly higher in Kinetic 12X (16%) and HiWett 24X (50%) treatments than in the other treatments. MSO 3X (15.5%) also had significantly higher damage than the water control (5%). Kinetic 12X and HiWett both contain organosilicones and when these types of adjuvants were used at very high rates, significant leaf injury resulted. Following the Jun-29 application in Albion, Kinetic 12X had significantly the highest bacterial bulb decay (39%), which was not significantly different than MSO 3X (24%). Following the Jul-13 application in Albion and the Jul-7 application in Elba, Kinetic 12X (Albion – 80%; Elba – 86%) and HiWett 24X (Albion – 67%; Elba – 91%) had significantly higher bacterial bulb decay than all other treatments, among which, there were no significant differences. A significant correlation (Pearson: $R = 0.5302$; $p = 0.0093$) occurred between leaf damage and bacterial bulb decay. ***These results suggest that when bacterial pathogens are present when leaf damage occurs, the incidence of bacterial bulb decay increases.*** No significant differences occurred among treatments for dry scale and there was no correlation between dry scale and leaf injury.

Single application of high rate of adjuvant without bacteria (Table 5). In Elba, when a single high rate of adjuvant was applied without bacteria, all adjuvant treatments had significantly higher bacterial bulb decay than water (12%), except LI700 3X (29%). HiWett 24X (75%) and Kinetic 12X (66.5%) had significantly higher bacterial bulb decay at harvest than all other treatments. Although damage was not recorded in the Elba trial, Kinetic 12X and HiWett 24X also caused considerable leaf injury as they did in the Albion trial. These results suggest that incidence of bacterial bulb decay increases with leaf injury. ***Perhaps other forms of leaf injury increase the incidence of bacterial decay? Factors that are known to cause leaf injury include hail, insect feeding from onion thrips and caterpillars, lesions from diseases such as IYSV and downy mildew, and phytotoxicity from herbicides (e.g. Buctril, Chateau). As an example, we know that the hail-injured onions often suffer great losses from bacterial decay.***

Effect of <u>multiple applications</u> of adjuvant prior to inoculation (Table 6 & 7). In Albion, the first two applications of adjuvants alone without bacteria were at the high rates, so the results were skewed towards the treatments that had the most damage, Kinetic 12X and HiWett 24X, also having had significantly the highest bacterial bulb decay. In Elba, there were no significant differences among treatments when adjuvant + bacteria were applied after two applications of standard rates of adjuvants or water alone. In general, bacterial bulb decay was very high for these treatments (trial average: 47%).

In Table 7, a single application of adjuvant with and without bacteria can be compared with two applications of adjuvants alone followed by an application with bacteria. For LI700, Kinetic, Dyne-Amic and HiWett, there were no significant differences between a single application of adjuvant with bacteria and without bacteria, although numerically, the application with bacteria was higher, except for LI700; and, when adjuvant + bacterial followed 2 applications of adjuvant alone, these treatments had 1.5X to 6.2X more bacterial bulb decay than the corresponding single treatments. Again, the application of an adjuvant or water without bacteria resulted in higher levels of bacterial bulb decay than back ground levels of decay. ***Also, multiple applications of adjuvant applied prior to inoculation with bacteria resulted in higher levels of bacterial bulb decay than single applications of adjuvant + bacteria. However, there were no significant differences between two application of water prior to water + bacteria and two applications of adjuvants prior to adjuvants + bacteria. Whether frequent use of adjuvants may elevate bacterial bulb decay requires further research.***

FURTHER RESEARCH:

Multiple applications of adjuvants both with and without bacterial inoculum are warranted to determine whether regular use of adjuvants (e.g. 5-8 sprays) in onion production increases the incidence of bacterial bulb decay in onions.

In light of the relationship between leaf injury and incidence of bacterial disease, both in inoculated and non-inoculated onions, further investigation of the relationship between leaf damage and bacterial bulb decay is warranted. Leaf damage caused by adjuvants and herbicide injury is of particular interest.

Given how relatively "easy" it was to incite bacterial bulb decay by spraying onions with a solution of bacteria in water, investigation of water sources (wells, ponds, ditches, creeks, cisterns, etc.) used for irrigating and spraying onions for pathogenic bacterial contamination is warranted.

RECOMMENDATIONS:

Growers are advised to exercise great caution in calculating rates and using adjuvants in their pesticide spray tank mixes, especially when using adjuvants that contain organosilicone-type adjuvants.

Be aware that not all adjuvants are used at the same rate. Kinetic and especially, HiWett are used at very low rates (e.g. 0.5 to 1.0 pints per 100 gal, compared to 4 pints per 100 gal for MSO & LI700).

If necrotic spotting/leaf burn is discovered on onion leaves and adjuvants have been used, consider that this injury may have been caused by an adjuvant, and review calculations and measuring procedures. Consider using a lower rate, another adjuvant or no adjuvant at all. Note that adjuvant injury is not easily distinguished from herbicide injury or the symptoms of certain leaf diseases.

Table 1. Adjuvants evaluated for their role in the development of bacterial diseases of onions, Hoepting *et. a.l.* 2012.

Trade Name	Company	Type of Adjuvant: Description	Active Ingredients	Standard Rates			High Rates		
				Rate in 80 gpa (40 gpa /pass)	Application Dates		Rate in 120 gpa (40 gpa /pass)	Application Dates	
					Albion	Elba		Albion	Elba
LI700	Loveland Products for CPS	**Non-ionic surfactant (NIS):** penetrant, acidifier, deposition aid, drift control agent	Phosphatidylchloine, methylacetic acid and alkyl phenol ethoxylate 80%	**0.5% v/v**[1] (=0.25% v/v, 2 passes)	Jul-26 Aug-9	Jul-20 Aug-3 Aug-9	**3x rate: 1.5% v/v** (=0.5% v/v, 3 passes)	Jun-29 Jul-13	Jul-7
Methylated Spray Oil (MSO)	Helena Chemical	**Methylated vegetable oil:** modifies wetting and deposition characteristics	alkyl phenol ethoxylate 100%	**0.5% v/v**[1] (=0.25% v/v, 2 passes)	Jul-26 Aug-9	Jul-20 Aug-3 Aug-9	**3x rate: 1.5% v/v** (=0.5% v/v, 3 passes)	Jun-29 Jul-13	Jul-7
Kinetic	Helena Chemical	**Organosilicone:** wetter/spreader/penetrant adjuvant	Proprietary blend of polyalkyleneoxide modified polydimethylsiloxane and nonionic surfactants 99%	**0.125% v/v**[1] (=0.0625% v/v, 2 passes)	Jul-26 Aug-9	Jul-20 Aug-3 Aug-9	**12x rate: 1.5% v/v** (=0.5% v/v, 3 passes)	Jun-29 Jul-13	Jul-7
Dyne-Amic	Helena Chemical	**Organosilicone & methylated vegetable oil blend:** Modified vegetable oil surfactant blend	Methyl esters of C16-C18 fatty acids, polyalkyleneoxide modified polydimethylsiloxane, alkylphenol ethoxylate 99%	**0.5% v/v**[1] (=0.25% v/v, 2 passes)	Jul-26 Aug-9	Jul-20 Aug-3 Aug-9	**3x rate: 1.5% v/v** (=0.5% v/v, 3 passes)	Jun-29 Jul-13	Jul-7
HiWett	Loveland Products for CPS	**Organosilicone blend:** Organosilicone surfactant blend with improved deposition to enhance spreading and deposition	Polysiloxane polyether copolymer, alcohol ethoxylate, polyoxyethylene-polyoxypropylene copolymer 100%	**0.0625% v/v**[1] (=0.25% v/v, 2 passes)	Jul-26 Aug-9	Jul-20 Aug-3 Aug-9	**24x rate: 1.5% v/v** (=0.5% v/v, 3 passes)	Jun-29 Jul-13	Jul-7

[1]4 pints per 100 gal = 0.5% v/v; 1 pt per 100 gal = 0.125% v/v; 0.5 pints per 100 gal = 0.0625% v/v.

Table 2. Spray conditions during each treatment application of adjuvant and bacteria, Albion and Elba, Hoepting *et al.*, 2012.

Trial Location:	**Albion: 'Candy' transplants (mineral soil)**				**Elba: 'Red Bull' direct seeded (muck)**			
Date:	**Jun-29**	**Jul-13**	**Jul-26**	**Aug-9**	**Jul-7**	**Jul-20**	**Aug-3**	**Aug-9**
Rates:	High	High	Standard	Standard	High	Standard	Standard	Standard
Crop stage:	8-9 leaf, pre-bulb	8-10 leaf, 1 inch bulbs	Na[1]	50% lodging	8 leaf, 1 inch bulbs	8-10 leaf, 1-2 inch bulbs	Na[1]	70-80% lodged
Start time:	1:00 pm	9:30 am	12:30 pm	11:15 am	7:30 am	2:36 pm	Na[1]	3:30 pm
Finish time:	2:15 pm	10:30 am	2:30 pm	12:05 am	8:46 am	3:22 pm	Na[1]	4:15 pm
Temperature:	92 ◦F	82 ◦F	85 ◦F	75 ◦F	77 ◦F	75 ◦F	Na[1]	86 ◦F
Relative humidity	41%	64%	69%	81%	73%	65%	Na[1]	54%
Wind speed (average):	3.8 mph	2.5 mph	6.3 mph	3.8 mph	3.5 mph	1.6 mph	Na[1]	2.0 mph
Wind speed (maximum):	7.4 mph	4.1 mph	10.9 mph	6.0 mph	5.5 mph	7.3 mph	Na[1]	3.6 mph
Nozzle type:	Single 8004 VS	Single 8004 VS	Single 8004 VS	Single 8004 VS	Single 8004 VS	Single 8004 VS	Single 8004 VS	Single 8004 VS
Nozzle spacing:	19 inches	19 inches	19 inches	19 inches	19 inches	19 inches	19 inches	19 inches
Spray Pressure:	28 psi	28 psi	28 psi	28 psi	28 psi	28 psi	28 psi	28 psi
Total spray volume:	3 x 40 = 120 gpa	3 x 40 = 120 gpa	2 x 40 = 80 gpa	2 x 40 = 80 gpa	3 x 40 = 120 gpa	2 x 40 = 80 gpa	2 x 40 = 80 gpa	2 x 40 = 80 gpa
Environmental conditions:	Hot, windy & sunny	Na[1]	Mostly sunny, windy, moist soil	Cloudy, overcast	overcast	Overcast, lot's of thrips, IYSV	Na[1]	Overcast, raindrops

[1]**Na:** information not available.

Table 3. Effect of <u>single application</u> of <u>standard rates</u> of adjuvants applied with bacteria inoculum on bacterial bulb decay and internal dry scale at harvest, Hoepting et al. 2012: Albion Trial.

Trial:	**Albion: 'Candy' transplants (mineral soil)**					
Crop Stage:	**Na**		**50% lodging**		**POOLED across all stages**	
Application Date:	**Jul-26**		**Aug-9**		**POOLED across all dates**	
Treatment (with bacteria)[1] and rate	**% bacterial rot at harvest**	**% internal dry scale[2] at harvest**	**% bacterial rot at harvest**	**% internal dry scale[2] at harvest**	**% bacterial rot at harvest**	**% internal dry scale[2] at harvest**
Water 80 gpa	22.0	2.7	8.0	3.5	15.0	3.0
LI700 0.5% v/v 80 gpa	15.9	2.0	3.9	0.7	9.9	1.4
MSO 0.5% v/v 80 gpa	11.8	2.3	5.9	0.7	8.9	1.5
Kinetic 0.125% v/v gpa	7.6	4.3	10.6	1.7	9.1	3.0
Dyne-Amic 0.5% v/v 80 gpa	7.3	0.0	5.0	0.6	6.2	0.3
HiWett 0.0625% v/v 80 gpa	8.9	3.2	8.6	1.6	8.8	2.4
P Value (α=0.05)	***NS[3]***	***NS***	***NS***	***NS***	***NS***	***NS***
Trial Average[4]	12.2%	2.4%	7.0%	1.5%	--	--
Background level[5]	6.1%	0.6%	6.1%	0.6%	6.1%	0.6%
Wound-Induced[6]	43.8%	12.5%	12.5%	12.5%	--	--
Time of inoculation	12:30 pm – 2:30 pm		11:15 am – 12:05 pm		--	
Conditions during inoculation	85◦F, 69% RH, mostly sunny, windy, moist soil		75◦F, 81% RH, over-cast		--	

[1]adjuvants applied with 1 x10[8] cfu/mL of bacterial pathogens of onions including *Burkholderia cepacia*, *Pantoea ananatis* and *Enterobactor cloacae* in solution.
[2]**internal dry scale:** inner scale just below neck is dry and not succulent; cause unknown. Bacterial pathogens not recovered from these internal dry scales.
[3]**NS:** Not significant, according to Fisher's Protected LSD test, p<0.05.
[4]**Trial Average:** average of all treatments and all reps.
[5]**background level:** 4 samples of onions were harvested from randomly selected 10-foot sections of row from the periphery of the trial area to determine natural or background levels of bacterial rot and dry scale.
[6]the leaves of 16 bulbs immediately in-front of the trial were moderately wounded using a forest frog before spraying water + bacteria. This was a test to see if the bacterial solution in water could induce infection when leaves were wounded.

Table 4. Effect of single application of standard rates of adjuvants applied with bacteria inoculum on bacterial bulb decay and internal dry scale at harvest, Hoepting et al. 2012: Elba Trial.

Trial:	**Elba: 'Red Bull' direct seeded (muck)**							
Crop Stage:	**7-9 leaf, 1-2 inch bulb**		**7-9 leaf, 2+ inch bulb**		**70-80% lodged**		**POOLED across all stages**	
Application Date:	**Jul-20**		**Aug-3**		**Aug 9**		**POOLED across all dates**	
Treatment (with bacteria)[1] and rate	**% bacterial rot at harvest**	**% internal dry scale[3] at harvest**	**% bacterial rot at harvest**	**% internal dry scale[3] at harvest**	**% bacterial rot at harvest**	**% internal dry scale[3] at harvest**	**% bacterial rot at harvest**	**% internal dry scale[3] at harvest**
Water 80 gpa	37.7	3.5	29.4	1.6	18.6 ab[2]	0.0	28.6	1.4
LI700 0.5% v/v 80 gpa	50.4	2.9	19.9	4.3	14.1 bc	2.3	28.4	2.6
MSO 0.5% v/v 80 gpa	39.0	0.0	15.3	0.5	7.9 c	1.4	20.7	1.0
Kinetic 0.125% v/v gpa	47.3	3.0	25.9	3.3	19.3 ab	1.4	30.8	2.3
Dyne-Amic 0.5% v/v 80 gpa	41.4	1.0	19.5	1.3	21.4 ab	2.3	25.8	1.9
HiWett 0.0625% v/v 80 gpa	47.0	2.5	17.1	4.5	28.2 a	3.9	30.8	2.9
P Value (α=0.05)	***NS[4]***	***NS***	***NS***	***NS***	***0.0146***	***NS***	***NS***	***NS***
Trial Average[5]	43.3%	2.3%	21.2%	2.6%	18.4%	1.9%	--	--
Background level[6]	11.3%	1.0%	11.3%	1.0%	11.3%	1.0%	11.3%	1.0%
Wound-Induced[7]	25.0%	0%	43.8%	6.25%	25.0%	0%	--	--
Time of inoculation	2:63 pm – 3:22 pm		Na[8]		3:30 pm – 4:15 pm		--	
Conditions during inoculation	75◦F, 65% RH, over-cast		Na[8]		86◦F, 55% RH, over-cast		--	

[1]adjuvants applied with 1 x10[8] cfu/mL of bacterial pathogens of onions including *Burkholderia cepacia*, *Pantoa ananatis* and *Enterobactor cloacea* in solution.
[2]Numbers in a column followed by the same letter are not significantly different, Fisher's Protected LSD test (p<0.05).
[3]**internal dry scale:** inner scale just below neck is dry and not succulent; cause unknown. Bacterial pathogens not recovered from these internal dry scales.
[4]**NS:** Not significant, according to Fisher's Protected LSD test, p<0.05.
[5]**Trial Average:** average of all treatments and all reps.
[6]**background level:** 4 samples of onions were harvested from randomly selected 10-foot sections of row from the periphery of the trial area to determine natural or background levels of bacterial rot and dry scale.
[7]the leaves of 16 bulbs immediately in-front of the trial were moderately wounded using a forest frog before spraying water + bacteria. This was a test to see if the bacterial solution in water could induce infection when leaves were wounded.
[8]**Na:** information not available.

Table 5. Effect of single application of high rates of adjuvants applied with and without bacteria inoculum on leaf damage, bacterial bulb decay and internal dry scale at harvest, Hoepting et al. 2012.

Trial:	Albion: 'Candy' transplants (mineral soil)					Elba: 'Red Bull' direct seeded (muck)			
	WITH BACTERIA[1]					WITH BACTERIA[1]		WITHOUT BACTERIA	
Crop stage:	8-9 leaf, pre-bulb			8-10 leaf, 1 inch bulbs		8-9 leaf, 1 inch bulb		8-9 leaf, 1 inch bulb	
Application date:	Jun-29			Jul-13		Jul-7		Jul-7	
Treatment (with bacteria)[1] and rate	**Leaf damage[2] on Jul 13**	**% bacterial rot at harvest**	**% internal dry scale[3] at harvest**	**% bacterial rot at harvest**	**% internal dry scale at harvest**	**% bacterial rot at harvest**	**% internal dry scale at harvest**	**% bacterial rot at harvest**	**% internal dry scale at harvest**
Water	4.7 bc[4]	11.5 b	0.0	10.9 b	1.4	53.2 b	1.6	11.7 c	1.6
LI700 3x 120 gpa	1.7 c	17.5 b	0.0	14.9 b	2.9	50.3 b	2.5	29.2 bc	1.6
MSO 3x 120 gpa	15.5 b	23.9 ab	0.28	12.0 b	1.3	59.6 b	2.1	34.7 b	2.1
Kinetic 12x 120 gpa	61.2 a	39.2 a	0.0	80.0 a	0.0	86.1 a	1.9	66.5 a	3.5
Dyne-Amic 3x 120 gpa	6.7 bc	9.1 b	0.65	18.0 b	3.6	58.5 b	1.9	31.1 b	2.6
HiWett 24x 120 gpa	49.7 a	18.6 b	0.75	67.3 a	0.9	90.6 a	1.3	74.9 a	1.9
P Value (α=0.05)	***0.0000***	***0.0096***	***NS[5]***	***0.0000***	***NS***	***0.0010***	***NS***	***0.0000***	***NS***
Trial Average[6]	23.3%	19.8%	0.2%	33.9%	1.7%	66.1%	1.9%	41.3%	2.2%
Background level[7]	na	6.1%	0.6%	6.1%	0.6%	11.3%	1.0%	11.3%	1.0%
Wound-induced[8]	na	na	na	37.5%	31.3%	68.8%	6.25%	81.3%	12.5%
Time of inoculation	1:00 pm – 2:15 pm			9:30 am – 10:30 am		7:30 am to 8:46 am		7:30 am to 8:46 am	
Conditions for inoculation	92◦F, 41% RH, hot, sunny, windy			82◦F, 64% RH		77◦F, 73% RH, overcast		77◦F, 73% RH, overcast	

[1]adjuvants applied with 1 x10^8 cfu/mL of bacterial pathogens of onions including *Burkholderia cepacia*, *Pantoea ananatis* and *Enterobactor cloacae* in solution.
[2]**leaf damage rating:** scale 0 to 100%; 0% = no damage, 100% = no green remaining.
[3]**internal dry scale:** inner scale just below neck is dry and not succulent; cause unknown. Bacterial pathogens not recovered from these internal dry scales.
[4]Numbers in a column followed by the same letter are not significantly different, Fisher's Protected LSD test ($p<0.05$).
[5]**NS:** Not significant, according to Fisher's Protected LSD test, $p<0.05$.
[6]**Trial Average:** average of all treatments and all reps.
[7]**background level:** 4 samples of onions were harvested from randomly selected 10-foot sections of row from the periphery of the trial area to determine natural or background levels of bacterial rot and dry scale.
[8]the leaves of 16 bulbs immediately in-front of the trial were moderately wounded using a forest frog before spraying water + bacteria. This was a test to see if the bacterial solution in water could induce infection when leaves were wounded.

Table 6. Effect of multiple applications of high and standard rates of adjuvants applied without bacteria prior to application with bacteria on bacterial bulb decay and internal dry scale at harvest, Hoepting et al. 2012.

Trial:	**Albion: 'Candy' transplants (mineral soil)**		**Elba: 'Red Bull' direct seeded (muck)**	
Adjuvant and Bacteria Applications:	**Jun-29 (high rates[8] w/o bacteria) Jul-13 (high rates[8] w/o bacteria) Jul 26 (std rates[9] w/o bacteria) Aug 9 (std rates[9] with bacteria[1])**		**Jul-20 (std rates[9] w/o bacteria) Aug-3 (std rates[9] w/o bacteria) Aug 9 (std rates[9] with bacteria[1])**	
Treatment	**% bacterial rot at harvest**	**% internal dry scale[2] at harvest**	**% bacterial rot at harvest**	**% internal dry scale[2] at harvest**
Water	8.8 c	1.1	43.9	2.8
LI700	15.3 bc	2.0	43.8	0.8
MSO	17.5 bc	0.0	48.5	0.7
Kinetic	49.2 a	0.0	50.6	0.6
Dyne-Amic	25.3 bc	2.3	47.8	2.3
HiWett	35.4 ab	2.4	46.2	1.4
P Value (α=0.05)	***0.0042[7]***	***NS[3]***	***NS***	***NS***
Trial Average[4]	24.1%	1.3%	46.8%	1.4%
Background level[5]	6.1%	0.6%	11.3%	0.6%
Wound-Induced[6]	--	--	56.3%	6.25%

[1]adjuvants applied with 1 x10^8 cfu/mL of bacterial pathogens of onions including *Burkholderia cepacia*, *Pantoea ananatis* and *Enterobactor cloacae* in solution.
[2]**internal dry scale:** inner scale just below neck is dry and not succulent; cause unknown. Bacterial pathogens not recovered from these internal dry scales.
[3]**NS:** Not significant, according to Fisher's Protected LSD test, p<0.05. [4]**Trial Average:** average of all treatments and all reps.
[5]**background level:** 4 samples of onions were harvested from randomly selected 10-foot sections of row from the periphery of the trial area to determine natural or background levels of bacterial rot and dry scale.
[6]the leaves of 16 bulbs immediately in-front of the trial were moderately wounded using a forest frog before spraying water + bacteria. This was a test to see if the bacterial solution in water could induce infection when leaves were wounded.
[7]Numbers in a column followed by the same letter are not significantly different according to Fisher's Protected LSD test, p<0.05.
[8]**high rates of adjuvants:** all 1.5% v/v 120 gpa; LI700 3X; MSO 3X; Kinetic 12X; Dyne-Amic 3X; HiWett 24X.
[9]**Standard rates of adjuvants:** all at 80 gpa; LI700 0.5% v/v; MSO 0.5% v/v; Kinetic 0.125% v/v; Dyne-Amic 0.5% v/v; HiWett 0.065% v/v.

Table 7. Effect of a single application of standard rates of adjuvant with and without bacteria, and the effect of multiple applications of adjuvant alone followed by a single application with bacteria, Hoepting et al. 2012 (Elba).

Elba: c.v. Red Bull direct seeded (muck)		
Adjuvant	**Treatment**	**% bacterial rot at harvest**
Water 80 gpa	Single app adjuvant + bacteria[1] (Aug-9)	18.6 cd[2]
	Single app adjuvant alone (Aug-9)	28.8 bc
	2 apps adjuvant alone (Jul-20 & Aug-3) plus adjuvant + bacteria[1] (Aug 9)	43.9 ab
LI700 0.5% v/v 80 gpa	Single app adjuvant + bacteria[1] (Aug-9)	14.8 cd
	Single app adjuvant alone (Aug-9)	18.1 cd
	2 apps adjuvant alone (Jul-20 & Aug-3) plus adjuvant + bacteria[1] (Aug 9)	43.8 ab
MSO 0.5% v/v 80 gpa	Single app adjuvant + bacteria[1] (Aug-9)	7.9 d
	Single app adjuvant alone (Aug-9)	28.1 c
	2 apps adjuvant alone (Jul-20 & Aug-3) plus adjuvant + bacteria[1] (Aug 9)	48.6 a
Kinetic 0.125% v/v 80 gpa	Single app adjuvant + bacteria[1] (Aug-9)	19.4 cd
	Single app adjuvant alone (Aug-9)	14.1 cd
	2 apps adjuvant alone (Jul-20 & Aug-3) plus adjuvant + bacteria[1] (Aug 9)	50.6 a
Dyne-Amic 0.5% v/v 80 gpa	Single app adjuvant + bacteria[1] (Aug-9)	21.4 cd
	Single app adjuvant alone (Aug-9)	16.6 cd
	2 apps adjuvant alone (Jul-20 & Aug-3) plus adjuvant + bacteria[1] (Aug 9)	47.8 a
HiWett 0.0625% v/v 80 gpa	Single app adjuvant + bacteria[1] (Aug-9)	28.2 c
	Single app adjuvant alone (Aug-9)	14.5 cd
	2 apps adjuvant alone (Jul-20 & Aug-3) plus adjuvant + bacteria[1] (Aug 9)	46.2 a
P Value (α=0.05)		***0.0000***

[1]adjuvants applied with 1 x10^8 cfu/mL of bacterial pathogens of onions including *Burkholderia cepacia*, *Pantoea ananatis* and *Enterobactor cloacae* in solution.
[2]Numbers in a column followed by the same letter are not significantly different according to Fisher's Protected LSD test, p<0.05.