Ecological Diversity, Niche Overlap, and Coexistence of Antagonists Used in Developing Mixtures for Biocontrol of Postharvest Diseases of Apples

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ABSTRACT

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A method was developed to select antagonists to be combined in mixtures that controlled blue mold of apple better than individual antagonists alone. Microorganisms selected for these mixtures were isolated from exposed apple tissue at weekly intervals over 5 weeks before harvest. The isolated microorganisms were classified and grouped into various nutritional clusters on the basis of their utilization of 95 carbon sources in Biolog plates using MicroLog and MLCLUST programs, respectively. Yeasts dominated isolations on nutrient yeast-dextrose agar at all isolation times. Bacteria were isolated only occasionally. Isolates were screened for their ability to control blue mold caused by *Penicillium expansum* on Golden Delicious apple fruit. The most promising antagonists from different clusters were paired in subsequent tests to control blue mold, with preference given to antagonists colonizing the same fruit, secondly to those colonizing different fruit, but isolated at the same time, and finally to those colonizing different fruit at different times of isolation. Among 21 yeast antagonists tested in 13 combinations, four combinations were superior to individual antagonists. In a more extensive test, control of blue mold by a combination of antagonist isolates T5-D3 and T5-E2 was consistently superior to the individual isolates. Nutritional profiles of these antagonists, based on utilization of 35 carbon and 33 nitrogen sources, revealed significant differences in carbon catabolism. These differences caused niche differentiation and allowed populations of both antagonists to flourish in the same wound. De Wit replacement series revealed a high level of coexistence between the two antagonists. This was further confirmed by the relative yield that was close to unity at all antagonist proportions tested. Combining antagonists on the basis of niche differentiation was an effective method of improving control of postharvest blue mold, and probably also for other pre- and postharvest diseases.

Fruits and vegetables suffer significant losses from parasitic diseases after harvest (4,7,35,36). Even a small loss can be very costly because of the accumulated value of growing, harvesting, and storing these high value commodities. Application of synthetic fungicides after harvest has been the main weapon in combating these diseases (8,9). However, the development of resistance to these fungicides among postharvest pathogens, a lack of replacement fungicides, and a public demand to reduce pesticide use created a need for alternative methods of control. Biological control of postharvest diseases has emerged in recent years as a promising alternative (11,16,19,28,39). Effective biological control has been reported for postharvest diseases of pome, stone, citrus, and various subtropical fruits (2,14,21,26,27,32). Recently, two bacteria (*Pseudomonas syringae*) and one yeast (*Candida oleophila* Montrocher) were registered by the Environmental Protection Agency in the United States for control of postharvest diseases of apple, pear, and citrus fruit. Efficacy and plasticity of the biocontrol system should be improved to make biocontrol effective under a wider range of conditions and against a greater number of pathogens at reduced cost. There are three main approaches to achieve this goal: i) improving the antagonist strain to increase its ecological fitness and biocontrol function, ii) changing the environment to the benefit of the antagonist, and iii) developing antagonist mixtures with superior biocontrol potential (19). Improvement of antagonist strains through genetic engineering has been criticized, because these products are consumed with food and work in this area has been delayed. However, strain improvement using "food grade" markers, such as bacteriocin and immunity markers, will be generally acceptable (3,19). Biological control of postharvest diseases has been improved by manipulating the nutritional environment of the antagonist. The addition of nutrients, such as L-asparagine and L-proline, enhanced the population of antagonistic *Pseudomonas syringae* at the wound site and improved biocontrol of blue mold of apple (24). The addition of the nutrient analog 2-deoxy-D-glucose (2-DOG) enhanced biocontrol of blue mold of apple by *Pseudomonas syringae* and *Sporobolomyces roseus* Kluyver & Niel (18,22). The 2-DOG reduced inoculum potential of *Penicillium expansum* Link by reducing glucose metabolism, which tilted pathogen/antagonist balance toward the antagonist. Recently, El Ghaouth et al. (10) suggested using this compound as a fungicide against a variety of postharvest diseases. The addition of $CaCl_2$ also had a beneficial effect on biocontrol of gray mold of apple (31).

Enhancing biological control by using mixtures of antagonists may have advantages: i) it may broaden the spectrum of activity, e.g., various fruit, cultivars, and maturity stages; ii) it may enhance the efficacy and reliability of the biological control; and iii) it allows the combination of various traits without employment of genetic engineering. Before antagonists that control both blue mold and gray mold of pome fruit were found, a combination of two antagonists, each effective against one of the two diseases, was used effectively to control both diseases at the same time (15). More recently, the combination of *Pseudomonas syringae* and pink yeast, *S. roseus*, each effective against both blue mold and green mold of apple, enhanced control of these diseases on mature apple fruit (20). This combination of antagonists was selected after evaluation of various randomly paired antagonist combinations, each effective against both diseases. In this paired

combination, populations of *Pseudomonas syringae* were not affected by the presence of *S. roseus*, but populations of *S. roseus* were affected by the presence of *Pseudomonas syringae*. We believed that superior microorganism mixtures could be selected if the selection is made using information about the ecology of the organisms inhabiting the fruit surface.

In this paper, we reported on the development of antagonist mixtures that were superior to individual antagonists in controlling postharvest blue mold of apple. The development of these mixtures was based on ecological knowledge of the antagonists' distribution in time, space, and nutritional niche differentiation.

MATERIALS AND METHODS

Isolation of the microorganisms. Five Golden Delicious apple trees (*Malus* × *domestica* Borkh.) were selected in a 6-year-old unmanaged orchard of Golden Delicious, Red Delicious, Empire, Stayman, and York apple trees at the Appalachian Fruit Research Station in Kearneysville, WV. Fruit on these trees were wounded at weekly intervals from 5 weeks to 1 week before harvest maturity. One wound per fruit was made with a sterile scalpel by re-

TABLE 1. Number of yeasts and bacterial species colonizing apple fruit tissue at various times before harvest

	Time of isolation (weeks before harvest)				
	4	3	2	1	0
Yeast	22[a]	18	16	11	10
Bacteria	4	3	3	1	1

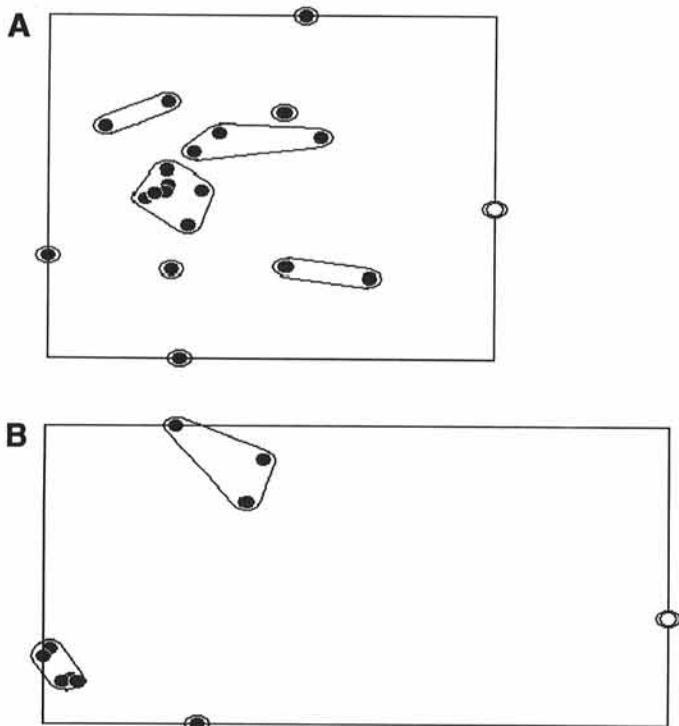[a] Number of species isolated at each interval.

Fig. 1. Two-dimensional presentation of cluster analysis of yeasts isolated from exposed tissue of Golden Delicious apple fruit in an unmanaged orchard: **A**, 5 weeks before harvest; and **B**, 1 week before harvest. The yeasts were isolated after 1 week of exposure of apple tissue. The isolates were classified using Biolog yeast (YT) plates and MicroLog program. Resulting data were subjected to cluster analysis using the MLCLUST program based on unweighted pair group method with arithmetic means. The distances between clusters indicate percent differences (*P* distances) in the utilization of 95 nutrients in Biolog YT plates. Each circle represents one or more isolates.

moving the skin about 2 mm deep on a 3 cm² area. The fruit samples were collected (one fruit from each of the five trees) 1 week after wounding, and isolation of microorganisms from the wounded area was made within 1 h. Tissue samples were removed from the wounded site with a cork borer (1 cm diameter × 1 cm deep). The resulting tissue cylinder was placed in a mortar with 1 ml of 0.05 M phosphate buffer at pH 6.5 (13), ground with a pestle, and dilution plated on nutrient yeast-dextrose agar (NYDA) medium (per liter: 8 g of nutrient broth, 5 g of yeast extract, 10 g of dextrose, and 15 g of agar). Thirty colonies were isolated per isolation time after a 5-day incubation period at 18°C, and, after triple restreaking, they were transferred to NYDA slants and stored under a phosphate buffer at 4°C for future use.

Classification and cluster analysis. Classification of the bacterial and yeast isolates was based on the Biolog system (Biolog Inc., Hayward, CA) using the utilization pattern of carbon sources available in gram negative bacteria (GN), gram positive bacteria (GP), and yeast (YT) plates. The cultures were activated on fresh slants and, after 24 h, were transferred to 250-ml Erlenmeyer flasks with 50 ml of nutrient yeast-dextrose broth (NYDB) medium. The flasks were placed on a rotary shaker at 150 rpm for 16 to 20 h. Cultures were then harvested by centrifugation at 8,000 rpm ($10,400 \times g$). After two washings in water, the cultures were adjusted to the appropriate concentrations using a spectrophotometer (Spectronic 21 DUV; Milton Roy Co., Rochester, NY) and starved for 1 h before application to the plates. Plates with yeast isolates were incubated for 72 h before reading with the microplate reader using the MicroLog-3N program (Biolog Inc.). The results from the YT plates for each isolation period and for all periods combined were subjected to cluster analysis using the MLCLUST program (Biolog Inc.) employing the unweighted pair group method with arithmetic means. Dendrograms, similarity matrices, and two- and three-dimensional plots were generated with this program.

The pathogen and microbial inocula. *P. expansum*, which was isolated from decaying apple, was grown on potato-dextrose agar. The conidia were collected from 10-day-old cultures and adjusted to the desired concentrations with the aid of a hemacytometer as described previously (25). The microorganisms for evaluation of biocontrol potential in mixtures and for replacement series were grown in NYDB, harvested by centrifugation, resuspended in water, and adjusted to desired concentrations as described above. There was no starvation period before application to the fruit.

Development of antagonist mixtures. To determine the biocontrol potential of the isolated microorganisms against *P. expansum*, each isolate was subjected to primary and secondary screening on Golden Delicious apples using methods described previously (17). Briefly, in the primary screening, each isolate was tested on nine fruits (three replications of three fruits, one wound per fruit). Twenty microliters of water suspension of the microorganisms was applied to each wound. The suspension was prepared by adding 24- to 48-h-old slant cultures to sterile water and adjusting turbidity (T) to 50% transmittance (0.3 optical density [OD]) at 420 nm. This was followed by applying 20 µl of conidial suspension of *P. expansum* (1×10^4 conidia/ml). The fruit were evaluated for rot development after 7 days of incubation at 24°C. Isolates causing reduction in wounds infected to less than 50% and inhibition of rot expansion by more than 75% were selected for secondary screening. In the secondary screening, fruit were treated similarly to primary screening, except that there were three concentrations of the antagonist (T of 95, 80, and 75%).

The most effective isolates from the secondary screening were used in biocontrol tests in various combinations. The effectiveness of these mixtures was compared with the effectiveness of the isolates applied individually. The combination of isolates selected for each of 13 mixtures were the most effective antagonists from

different nutritional clusters and isolated from i) the same fruit; ii) different fruit, but at the same time; or iii) different fruit at various times. Thirteen combinations of 21 isolates were tested. The efficacy of the mixtures and individual isolates was evaluated in drop inoculation factorial experiments conducted on wounded Golden Delicious apple fruit (two wounds per fruit) according to an earlier described procedure (23).

There were three concentrations of the antagonists (T of 95, 80, and 75% transmittance at 420 nm, corresponding to 0.02, 0.1, and 0.12 OD) ranging approximately from 1×10^6 to 5×10^7 and 5×10^7 to 1×10^9 CFU/ml for yeasts and bacteria, respectively; and three concentrations of *P. expansum* conidia (1.25, 2.5, and 5×10^5 conidia/ml). Mixtures of the antagonists were prepared by mixing 1:1 vol/vol suspensions used for individual applications at given turbidity. The treatments were arranged in randomized blocks and there were three replications of a single fruit with two wounds per treatment. After these tests, four antagonist mixtures that showed enhanced control over individual isolates were selected for additional tests. These tests were almost identical to the earlier ones, except that concentrations of *P. expansum* conidia were 1, 5, 10, and 50×10^4 conidia/ml, and there were nine fruits per replication. In control treatments, water was applied, instead of antagonist suspension, before inoculation with *P. expansum* conidia.

Replacement series for antagonists in the mixture. To determine if there was competition for nutritional resources in apple wounds between populations of the antagonists in the mixture, we conducted tests similar to the de Wit replacement series (5) adopted to microbiological applications (40,41).

Yeast isolates from the most effective mixture (isolates T5-D3 and T5-E2) were grown in NYDB, harvested by centrifugation, and the concentration was adjusted to T of 75% as described above. The suspensions of the two isolates were mixed at various proportions (vol/vol) from 1:0 to 0:1 in 0.2 increments. Also, suspensions of individual isolates were mixed with water in the above proportions. Golden Delicious apples were surface sterilized with 70% ethanol 1 day before application of the antagonist. The fruit were wounded by removal of tissue blocks approximately $3 \times 3 \times 3$ mm, and 25 µl of the suspension was placed in the wound.

Recovery of the antagonists was conducted just after their application when there was no more free suspension in the wound and after 24 and 48 h. To recover antagonists, the wounded area was removed with a cork borer (1 cm in diameter) to a depth of 1 cm. The resulting cylinder was placed in a Stomacher bag (Seward Medical, London) with 4.5 ml of 0.05 M phosphate buffer (pH 6.5) and blended in a Stomacher 80 blender (Seward Medical) for 2 min at normal speed. The resulting slurry was filtered through glass wool, and the suspension was diluted 10-fold and plated on NYDA media. The colonies were counted after approximately 48 h of incubation at 24°C, which allowed for visual differentiation based on color and size of the colonies (T5-E2, light pink and smaller; T5-D2, whitish and larger) of the two yeasts in isolations from mixed applications.

Nutritional profile and niche overlapping. Nutritional profile tests of the two yeast isolates, T5-D3 and T5-E2, were conducted in microtiter plates in a minimum salt (MS) medium containing various carbon or nitrogen sources and a vitamin supplement. The procedure was similar to the one described previously, except that the composition of the medium was different (24). The MS medium contained the following per liter: 0.85 g of KH_2PO_4, 0.15 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NaCl, 0.1 g of $CaCl_2 \cdot 6H_2O$, 500 µg of H_3BO_3, 40 µg of $CuSO_4 \cdot 6H_2O$, 200 µg of $FeCl_3 \cdot 6H_2O$, 400 µg of $MnSO_4 \cdot 4H_2O$, 200 µg of $Na_2MoO_4 \cdot 2H_2O$, 400 µg of $ZnSO_4 \cdot 7H_2O$, and vitamins. The vitamin requirements of the yeast isolates were determined according to the procedure described by Barnett et al. (1). Isolate T5-D3 required biotin (20 µg/liter), myo-inositol (10 mg/liter), nicotinic acid (400 µg/liter), pantothenic acid (2 mg/liter), and isolate T5-E2 biotin for growth in MS medium. Nutritional profiles were also determined from

Biolog YT plates (Biolog Inc.) for both yeasts. The niche overlapping index (NOI) (NOI = number of carbon or nitrogen sources utilized in common by both antagonists/total number of carbon or nitrogen sources utilized by one [targeted] antagonist) and the relative yield (RY) (RY = population size when coinoculated with other antagonist/population size when inoculated alone) were calculated according to procedures described by Wilson and Lindow (40,41).

Data analysis. Analysis of variance was performed on rot development data from the fruit treated with antagonist T5-D3 and T5-E2 individually and in a mixture, with the general linear models procedure of the Statistical Analysis System based on type III sums of squares of balanced linear model and randomized block design (34). A Waller-Duncan (*K* ratio = 100) multiple range test was performed for separation of means of lesion diameter and percentage of wounds infected.

RESULTS

Diversity of microorganisms colonizing on exposed apple tissue. Yeast populations on exposed apple tissue were higher at all isolation times; bacteria were isolated only occasionally. As fruit approached harvest maturity, the number of yeast and bacterial species declined; yeast declined from 22 species at 4 weeks before harvest to 10 species at harvest, and bacteria declined from four species at 4 weeks before harvest to one species at harvest (Table 1).

Cluster analysis of the yeast communities, based on the utilization pattern of nutrients in Biolog YT plates (Biolog Inc.), revealed a declining trend in the number of clusters (Fig. 1A and B). The number of clusters declined from four clusters at 4 weeks before harvest, to three clusters at 3 and 2 weeks before harvest, to only two clusters at 1 week before and at harvest. Cluster analysis of yeast isolates combined from all isolation times revealed four major and four small clusters (Fig. 2). The largest cluster (black) contained representatives from all five isolation
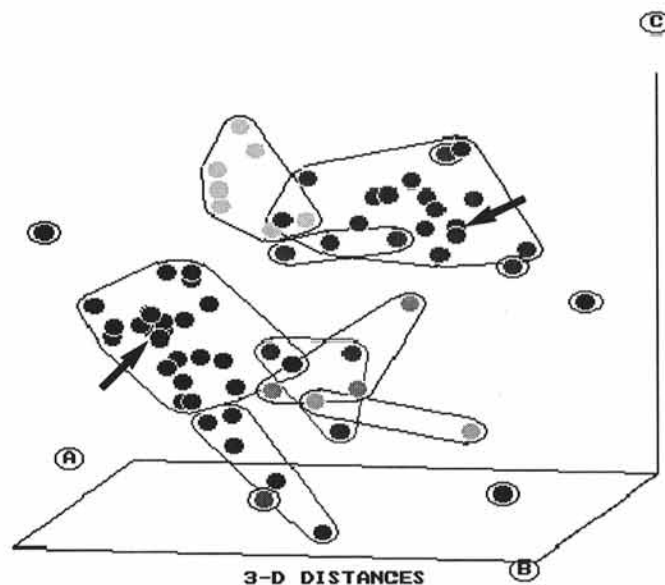
Fig. 2. Three-dimensional presentation of cluster analysis of yeasts isolated from exposed tissue of Golden Delicious apple fruit in an unmanaged orchard during the last 5 weeks before harvest. The samples were collected at weekly intervals after exposing apple tissue for 1 week. The isolates were classified using Biolog yeast (YT) plates and MicroLog program. The data from all isolation times were combined and subjected to cluster analysis using the MLCLUST program based on unweighted pair group method with arithmetic means. The distances between clusters indicate percent differences (*P* distances) in the utilization of 95 nutrients in Biolog YT plates. The identity of the isolates was removed for clarity. The arrows indicate antagonists T5-D3 (lower arrow) and T5-E2 (upper arrow).

times, the second largest (darkest shade) from the last four isolations, and the third largest (light shade at the top) only from the first two isolations.

Biocontrol potential of the isolates. Yeasts that could control blue mold were present at all isolation times (Table 2). The yeast

populations at harvest had the highest percentage of isolates that controlled blue mold most effectively. All four major clusters contained yeasts with good biological control potential.

Antagonist mixtures. From 21 yeast antagonists tested for control of blue mold of apple in 13 different mixtures, control by four mixtures was distinctively better than that of the individual antagonists. This improvement was observed for both the severity (lesion size) and incidence (percentage of wounds infected) of the disease (rots). More extensive tests with the four superior combinations revealed that isolates T5-D3 and T5-E2 gave consistently better control in a mixture than in individual applications. Treatment with these antagonists had significant effects on the severity ($P = 0.0001$) and incidence ($P = 0.0001$) of rots. Fruit treated with the mixture of these antagonists had smaller lesion size and lower incidence of blue mold than fruit treated with the individual antagonists ($P = 0.05$). The concentration of the antagonists and the pathogen also significantly affected the severity ($P = 0.0026$ and $P = 0.0001$, respectively) and incidence ($P = 0.0067$ and $P = 0.0001$, respectively) of blue mold. As the concentration of the antagonists increased, the severity and incidence of the rots decreased ($P = 0.05$) (Figs. 3 and 4). As the concentration of the pathogen increased, blue mold severity and incidence increased ($P = 0.05$). Among control treatments, the percentage of wounds infected was within the range of 83 to 100%, and lesion diameter was within the range of 20 to 34 mm at all four concentrations of *P. expansum* conidia.
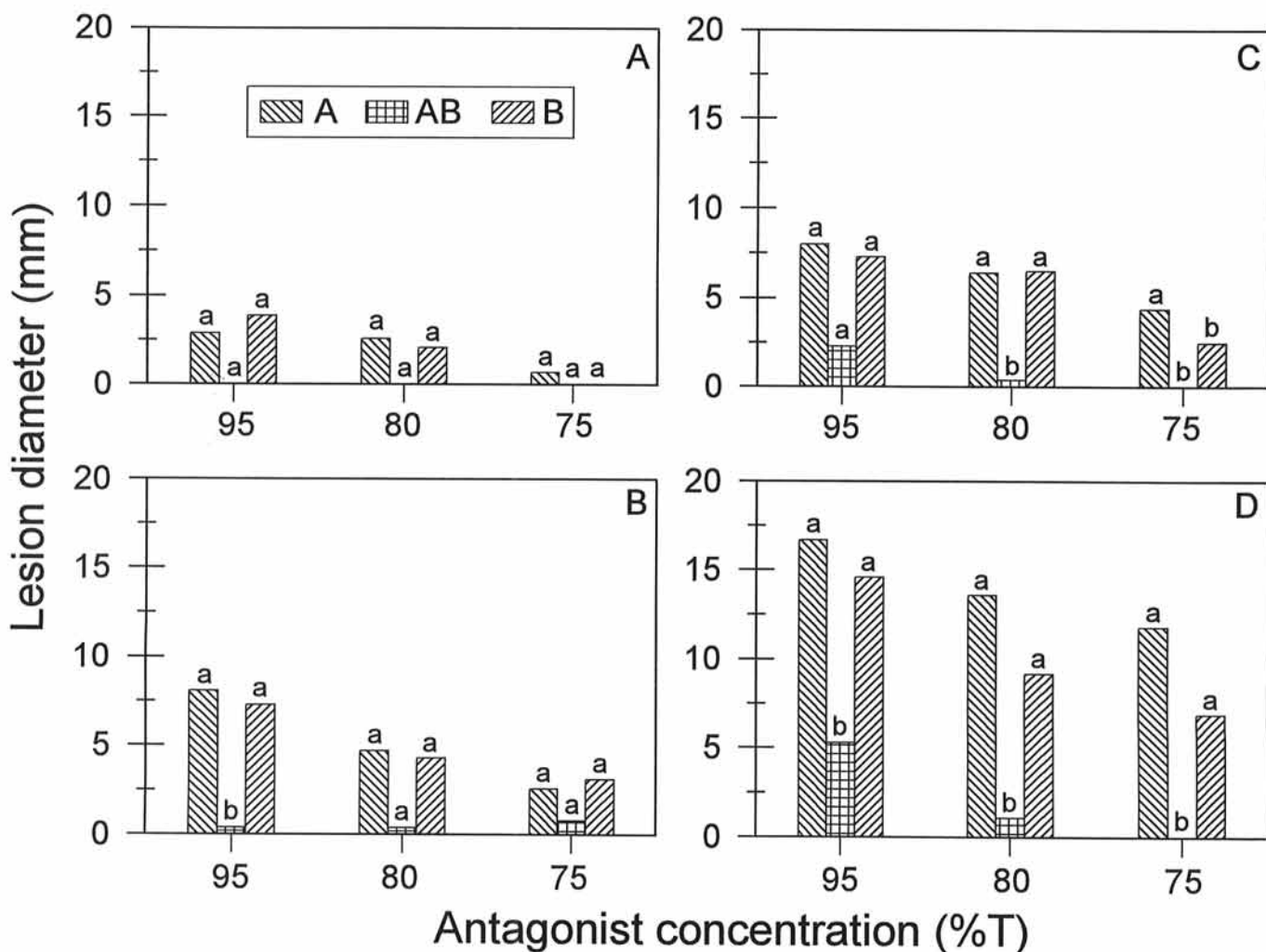
TABLE 2. Biocontrol potential of yeasts colonizing apple fruit tissue at various times before harvest against *Penicillium expansum* on Golden Delicious apples (primary screening)

Number of wounds infected with *P. expansum*[a]	Time of isolation (weeks before harvest)				
	4	3	2	1	0
0	31.0[b]	12.5	23.8	33.3	46.1
1	13.8	12.5	14.3	11.1	0.0
2	10.3	16.7	9.5	11.1	0.0
3	10.3	20.8	0.0	11.1	30.3
4	3.4	8.3	4.8	11.1	7.7
5	6.9	8.3	9.5	11.1	0.0
6	0.0	8.3	23.8	11.1	7.7
7	10.3	0.0	0.0	0.0	7.7
8	3.4	4.2	9.5	0.0	0.0
9	10.3	8.3	4.8	0.0	0.0

[a] Golden Delicious apples were wounded (three fruit for each of three replications, one wound per fruit), inoculated with aqueous suspension of yeasts (50% transmittance or optical density 0.3 at 420 nm), and challenged with conidia of *P. expansum* (1×10^4 conidia/ml). Number of wounds infected was determined after incubation for 7 days at 22°C.

[b] Percentage of isolates from a given time of isolation.

Fig. 3. Lesion development on wounded Golden Delicious apple fruit protected with antagonists T5-D3 (A) and T5-E2 (B) applied alone or in mixture (AB) and challenged with *Penicillium expansum* at concentrations of **A,** 1×10^4; **B,** 5×10^4; **C,** 1×10^5; and **D,** 5×10^5 conidia/ml. The mixtures were made by combining equal volumes of suspensions of each antagonist. Bars with the same letters are not significantly different, according to a Waller-Duncan multiple range test, *K* ratio = 100. %T = percent turbidity.

Coexistence of the T5-D3 and T5-E2 antagonists. Populations of both antagonists, applied individually or in mixtures, increased during the first 24 h after application to the wounds of Golden Delicious apples. No additional increase in populations of the isolates was observed at 48 h after application, indicating that populations had reached an equilibrium at 24 h. Therefore, only the results from the 24-h isolation are presented. De Wit replacement series revealed no significant influence of the presence of one antagonist on the population of the other at all ratios tested (Fig. 5). The mean RY of both antagonists approximated 1 at all ratios tested (Table 3).

Nutritional profile and niche overlap. From 35 carbon sources tested in MS medium, T5-E2 utilized 15 and T5-D3 utilized four: glucose, fructose, mannose, and cellobiose. All four carbon sources utilized by T5-D3 were also utilized by T5-E2, resulting in a NOI of 1 for the antagonist T5-D3 (Table 4). The NOI for T5-E2 was only 0.267. Of the three major soluble sugars occurring in apple (glucose, fructose, and sucrose), T5-D3 utilized only glucose and fructose; T5-E2 utilized all three sugars. The magnitude of differences between the two antagonists in NOI of 95 carbon sources included in the Biolog plates (Biolog Inc.) was similar to the 35 carbon sources tested in the MS medium. From 33 nitrogen sources tested in MS medium, T5-D3 utilized 26 and T5-E2 utilized 23. Twenty-two of the nitrogen sources were utilized by both antagonists. This resulted in similar NOI for both antagonists. The nitrogen source utilized exclusively by T5-

E2 was L-cysteine; the sources used exclusively by T5-D3 were acetamide, taurine, choline, and L-valine. Six nitrogen sources, sodium nitrate, glucosoamine, creatine, trans-4-hydroxyl-L-proline, 2-aminoisobutric acid, and n-ethylamaleimide, were not utilized by either antagonist.

DISCUSSION

Combining yeast antagonists with different nutritional profiles resulted in increased control of blue mold, as compared with treatments containing the individual isolates. Consistently, superior control by the combination of T5-D3 and T5-E2 compared with the individual antagonists indicated the usefulness of antagonist mixtures in improving biological control efficacy. Improved biological control with antagonist mixtures has been reported in other systems. In a soil system, for example, control of Fusarium wilt was improved by combining an antagonistic, nonpathogenic strain of *Fusarium oxysporum* with *Pseudomonas putida*, which did not reduce disease severity on its own (29). Competition for limited glucose was the mechanism in this system. Pseudobactin produced by *Pseudomonas putida* reduced efficiency of glucose metabolism to a greater degree in a pathogenic than in a nonpathogenic strain of the fungus, giving competitive advantage to the latter (30). In our studies, only those yeasts having strong biocontrol potential were combined, because the main mechanisms of biocontrol by these yeasts were thought
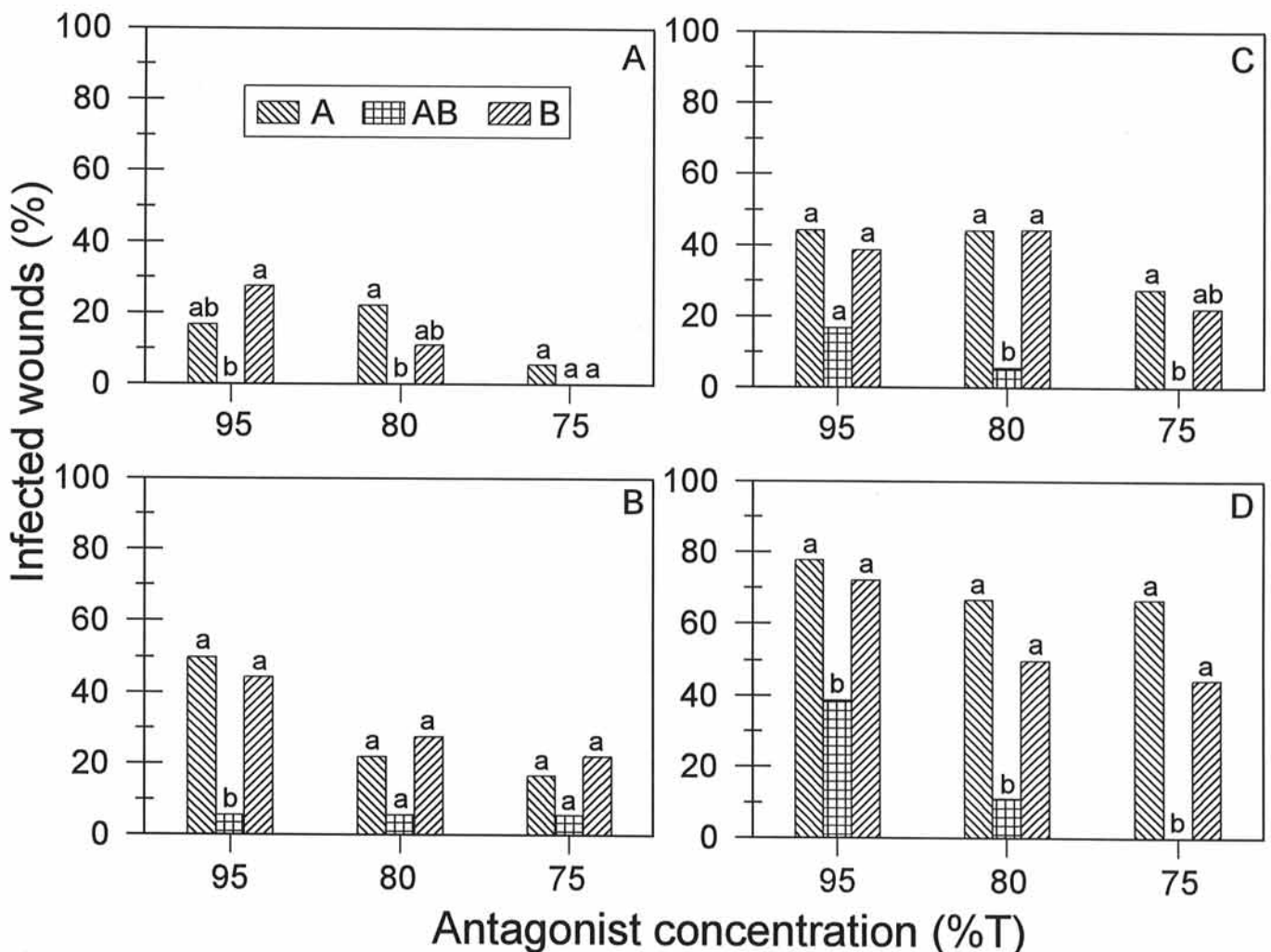

Fig. 4. Percentage of infected wounds on Golden Delicious apple fruit protected with antagonists T5-D3 (A) and T5-E2 (B) applied alone or in mixture (AB) and challenged with *Penicillium expansum* at concentrations of **A**, 1×10^4; **B**, 5×10^4; **C**, 1×10^5; and **D**, 5×10^5 conidia/ml. The mixtures were made by combining equal volumes of suspensions of each antagonist. Bars with the same letters are not significantly different, according to a Waller-Duncan multiple range test, K ratio = 100. %T = percent turbidity.

to involve direct competition for nutrients and niche exclusion (6,23). However, considering the above example, the possibility of improving biocontrol of fruit rots by combining antagonists with microorganisms that are not effective alone cannot be completely excluded.

Grouping of microbial populations into clusters (guilds) with catabolically similar species can be useful in characterizing microbial communities, studying nutritional relationships in the communities, and determining microbial interactions on aerial plant surfaces (24,40,41). In our studies, it was useful in selecting mixtures of mutually compatible antagonistic yeasts with superior biological control potential to the individual isolates. The high level of coexistence of such selected yeasts was demonstrated in de Wit replacement series experiments, in which populations of both antagonists increased more than 10-fold and populations of one were not affected by the presence of the other. This was further confirmed by a RY value close to 1 for both yeasts at all ratios tested. The clustering also revealed declining diversity among yeast, since the number of nutritional (carbon utilization based) clusters declined as harvest approached. Physiological changes associated with the maturation of apple fruit were most likely responsible for this decline. Soluble sugars, such as glucose, fructose, and sucrose, increase during maturation and may have created an environment favorable for some yeasts, which became dominant closer to harvest (12,37,38). However, more studies are needed to confirm this observation. In our earlier work, two biocontrol agents, *S. roseus* and *Pseudomonas syringae*, which in combination were more effective against blue mold of apple than individually, had different nutritional profiles (20). There was apparently little competition between the antagonists in apple wounds, since populations of *Pseudomonas syringae* were not affected by the presence of *S. roseus*, and populations of *S. roseus* were only slightly lower in combination than in individual applications. In biological control of frost injury, similarity of near-isogenic Ice– and Ice+ *Pseudomonas syringae* strains in utilizing various carbon sources explained equal competitiveness of the strains for the limited resources on the plant surface and effective exclusion of the Ice+ strain by precolonization of the plant surface with the Ice– strain (40). Thus, knowledge of nutritional profiles of microorganisms may be helpful in predicting their interaction on plant surfaces and warrants further research in this area.

The scarcity of the bacteria in these studies may be the result of strong competition from the yeasts, which thrive on substrates rich in available carbon, and/or dry conditions during the isolation period, which may have favored yeasts because they can endure much drier conditions than bacteria (33).

Niche differentiation estimated from in vitro carbon utilization profiles has been used as an indicator of ecological coexistence of microorganisms on plant surfaces (41). NOI for 35 carbon sources, which we selected, and 95 carbon sources in Biolog plates (Biolog Inc.) were similar, confirming earlier reports on the usefulness of Biolog plates (Biolog Inc.) in determining niche differentiation (40). T5-D3 had a low NOI with respect to T5-E2, but T5-E2 had a high NOI with respect to T5-D3, when carbon utilization profiles were used. However, the fact that T5-D3 utilized only four carbon sources out of 35 tested in MS media, two of which were glucose and fructose (the major soluble sugars occurring in apple), suggested great specialization and the probable efficacy of this antagonist in the utilization of these carbon sources. In contrast, T5-E2 utilized 15 carbon sources, which included all three major soluble sugars occurring in apple. Thus, the inability of T5-D3 to utilize sucrose, combined with efficient utilization of glucose and fructose by this yeast, may have resulted in niche differentiation that allowed both antagonists to flourish. To confirm this, tests should be conducted on partitioning of glucose, fructose, and sucrose between these two yeasts.

Our results indicated that efficacy of biological control of postharvest diseases can be improved greatly by using antagonist mixtures, but, more importantly, that combining antagonists that occupy different nutritional niches and coexist in the infection court are more effective biological control treatments than individual antagonists.
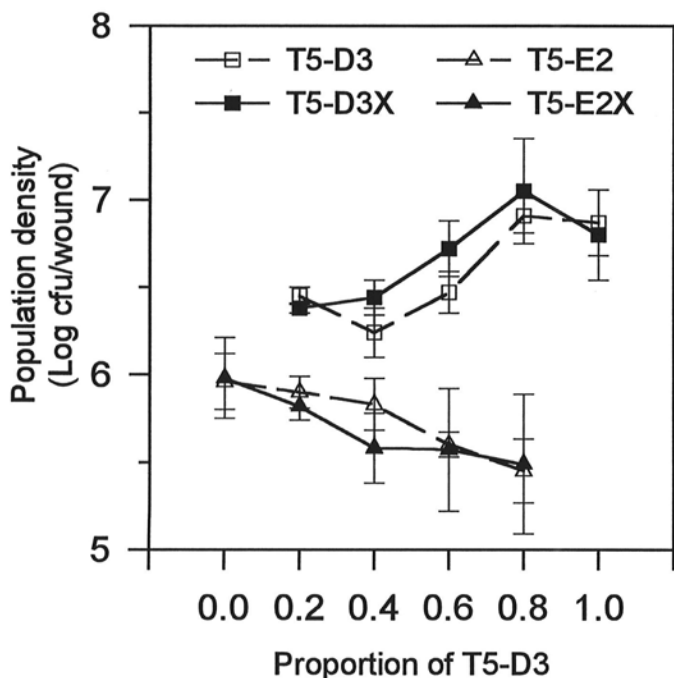

Fig. 5. Coexistence of yeast antagonists T5-D3 and T5-E2, mixed at various proportions, 24 h after application to the wounds of Golden Delicious apple fruit. The dashed line represents populations of the antagonists inoculated alone (in this treatment, water was added instead of the other antagonist), and solid lines indicate the populations when coinoculated at various proportions (T5-D3X and T5-E2X). The bars indicate ± 1 SD.

TABLE 3. Levels of coexistence between antagonists T5-D3 and T5-E2 in apple fruit wounds determined from replacement series experiments

Antagonist ratio	Mean[a] relative yield	
	T5-D3	T5-E2
1:0	0.9884	1.0034
0.8:0.2	1.0203	0.9864
0.6:0.4	1.0386	0.9571
0.4:0.6	1.0354	0.9946
0.2:0.8	0.9891	1.0073

[a] Levels of coexistence of one strain with respect to the other determined from the means of three samples for each inoculum proportion. The relative yield (RY) represents the ratio of population size when coinoculated to population size when inoculated alone. A mean RY of approximately 1 indicates complete coexistence of one antagonist with the other.

TABLE 4. Niche overlap index (NOI) for two antagonists derived from carbon and nitrogen sources utilization data

Targeted antagonist	NOI[a]		
	Individual carbon sources	Individual nitrogen sources	Biolog plate[b]
T5-E2	0.267	0.846	0.141
T5-D3	1	0.956	0.9

[a] NOIs were derived from carbon and nitrogen utilization in minimum salt (MS) medium supplemented with individual carbon (36) or nitrogen (33) sources. NOI = number of nutrient (carbon or nitrogen) sources utilized in common by both antagonists/total number of nutrient (carbon or nitrogen) sources utilized by one (targeted) antagonist.
[b] Biolog plate contained 95 carbon sources.

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